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But the Society being of late years greatly enlarged, and their communications more numerous, it was thought advisable that a Committee of their members should be appointed to reconsider the papers read before them, and select out of them such as they should judge most proper for publication in the future *Transactions;* which was accordingly done upon the 26th of March, 1752. And the grounds of their choice are, and will continue to be, the importance and singularity of the subjects, or the advantageous manner of treating them; without pretending to answer for the certainty of the facts, or propriety of the reasonings contained in the several papers so published, which must still rest on the credit or judgment of their respective authors.

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(B.)

VOL. 185.—PART I.

LIST OF ILLUSTRATIONS.

PHILOSOPHICAL TRANSACTIONS.

I. *Upon the Existence of more than one Fungus in Madura Disease (Mycetoma).*

By Rubert Boyce, *M.B., Assistant Professor of Pathology, University College, London, and* Nusserwanji F. Surveyor, *M.D., M.R.C.P.*

(From the Pathological Laboratory of University College, London.)

Communicated by Professor Victor Horsley, *F.R.S.*

[Plates 1–4.]

The scientific history of the fungus disease of India dates from the publication, in 1874, of the collected papers of Vandyke Carter. This observer showed that the fungus foot was a "veritable parasitic disease, due to the growth and extension within the tissues of the human foot of an indigenous mould." He demonstrated the presence of the parasite in all specimens examined, and came to the conclusion that it was one species, the *Chionyphe Carteri*.

In 1888 new observations were published in the collected papers of Lewis and Cunningham. They came to the conclusion that mycetoma was "essentially a degeneration of the fatty tissues, independent of the local presence or influence of any parasites whatever."

In 1888, Bassini* described a case of the black variety of the fungus in Italy (the first case in Europe), and by means of caustic potash concluded that the organism was a mycelial fungus, somewhat of the nature of the *Aspergilli* or *Mucorini*.

Lastly, in 1892, Dr. Kanthack† re-asserted the fungoid nature of the disease, and brought forward evidence to show the identity, or close affinity, of the organism with that of *Actinomycosis*. We, on the other hand, will bring forward reasons for believing that there are at least two distinct fungi—one a very highly organised species, the other a very delicate and lowly organised type, presenting very many of

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* Bassini, 'Bact. Centrlb.,' 1888.

† 'Journal of Pathology and Bacteriology,' No. 2.

the characters of *Actinomyces*. And we further think that we will be able to account for the apparent discrepancies in observation in the case of the previous writers.

The Madura foot of India is a very chronic affection, lasting in some cases twenty-five years.* It is a purely local disease of the extremities, chiefly the foot, and generalisation has not been observed. It usually occurs in people who go barefoot and are working in fields. In most cases it has been traced to some injury. The big toe is often affected at first. The disease stops at the ankle for a short time, then it spreads up to the knee, and eventually may even reach the thigh. One of us has had the opportunity of seeing a recurrence in the scar after amputation ; this, however, must be very rare, and amputation affords complete relief—and is, indeed, one of the most successful operations in India. The foot, as the photograph 1 shows, is greatly altered; it is enlarged, often many times the natural size. The overgrowth of the foot is irregular ; the toes may become buried, as shown in the figure, and the surface become studded over with mammillated, or even villous, projections. A large number of the mammillated projections mark the presence of sinuses, which pass deeply into the foot ; and on section (fig. 2) these may be seen to honeycomb it. From the opening of the sinuses a purulent or sanious discharge can be pressed out, and in this are found, in one series of cases, small particles of a light yellow colour, which have been compared to fish roe ; whilst, in the remaining cases, deep brown or black particles, resembling grains of gunpowder, may be seen. The disorganisation of the interior of the foot becomes very complete in time ; the bones undergo a rarefactive osteitis, and are ultimately absorbed ; granulation tissue also invades the muscles and fat, and leads to their disappearance. Associated with the hypertrophy of the granulation tissue there may be considerable hyperplasia of the epithelium of the skin. This overgrowth gives rise to the mammillated and papillomatous projections previously referred to.

The only difference which one at present finds, clinically and microscopically, in the numerous cases of Madura foot is the difference in the size and colour of the particles. CARTER termed that form of the disease in which the black particles were present the "melanoid" variety, while under the "white" or "ochroid" were grouped those cases in which the fish roe-like bodies were found. It will be understood that only those cases of Madura disease in which the *particles* can be demonstrated macroscopically or microscopically will be admitted as genuine. The disease appears to be confounded with scrofula and various forms of elephantiasis, in the production of which probably other parasites play an important part. In this paper we will confine ourselves chiefly to the elucidation of the fungus of the melanoid variety, and contrast it with that of the white. This is all the more necessary, because the older writers, from the time of VANDYKE CARTER, agreed that the black bodies did show some kind of vegetable structure, whilst the structure of the white appeared very obscure. Yet the most recent writer upon the subject, Dr. KANTHACK, takes the opposite view,

* NUSSERWANGI F. SURVEYOR, "Madura Foot of India." 'Brit. Med. Journ.,' Sept., 1892.

and in the three melanoid specimens which he examined he "found it difficult to convince himself of their vegetable nature."

On examining a diseased foot, the black particles will be found in abundance, both loose in the sinuses and packed closely in cavities in the tissue. A fresh section with the razor may cut across a black mass firmly imbedded in the tissue, and the intimate relationship of both can then be readily seen with the naked eye. The particles vary very greatly in size, from a pin's head, or even smaller, to the large mass three-quarters of an inch in diameter, depicted in fig. 3. In shape they are irregular, and a dendritic form can very often be seen. Fig. 3 has the mulberry form which is often described. On section, the appearance is somewhat radiate. Fig. 4 is a section through the large mass (fig. 3), slightly magnified (Obj. 35 mm., ZEISS). Fig. 5 passes through a minute particle in another specimen; it is more highly magnified (Obj. D, ZEISS), and the appearance is distinctly radiate, but this fine radiation is not the same as the coarse radiate grouping seen with the naked eye, and in the preceding figure. As regards colour, the naked eye examination shows that the particles are darkest at the periphery, becoming a lighter brown as the centre is approached. Examinations with the immersion, as in fig. 6, seem at first sight to throw very little light upon the nature of these curious black masses; a slight venation may be seen, as in fig. 6, or peculiar holes in a homogeneous matrix, as in figs. 5 and 6A. It was in these apparently structureless masses that we first demonstrated the existence of a well-formed, large-branching, and septate fungus. The method of procedure was as follows :—
A black particle was boiled for from one minute to half an hour in concentrated caustic potash; the particle, which had undergone very little apparent change, was next transferred to distilled water, when all the black colouring matter rapidly diffused out, leaving behind a white soft mass. This, upon microscopic examination, proved to consist wholly of a fungus similar to that represented in figs. 7–9. A segment of the large black mass (fig. 3) was, after similar treatment with caustic potash and water, dehydrated and imbedded in collodion; sections subsequently revealed a similar fungus. Caustic potash, we found, had been used by other observers. Thus, LEWIS and CUNNINGHAM direct that a crushed particle should be placed for a considerable time in a test-tube of strong caustic potash; a sediment collects at the bottom which equals the $\frac{1}{10}$th of the original bulk; upon microscopic examination, this proves to consist of hyphæ not distinguishable from those of fungi. From an illustration of the hyphæ given by the authors, it appears they are identical with those we find. They, however, look upon these elements as unimportant constituents of the black masses, which they think have an origin identical with the other pigmentary deposits in animal tissues. The fungus is to them a mere "epiphenomenon" in the particle. In our method of treatment with boiling caustic potash and water, we in no way disturb the arrangement of the fungus, and we have succeeded in washing and mounting sections of particles as small as a pin's head. The method, however, has the disadvantage that it did not permit us to examine the decolourized fungus *in situ*

in the tissues, any trace of animal tissue being destroyed by the concentrated caustic potash, nor does it permit the use of collodion. We had at this stage to content ourselves with the examination of the brown amorphous masses in the tissues, and of the decolourized particles after removal from them.

Professor OLIVER,[*] however, brought to our notice the use of *Eau de Javelle* as a clearing re-agent superior to caustic potash for vegetable structure. Its application in our case was most successful. If the black particles are dehydrated, embedded in collodion, and cut, and the sections are then steeped in the *Eau de Javelle* for from two to five minutes, or until gas bubbles begin to appear in them, it will be found that the fungus has been rendered beautifully transparent, whilst remains of the original animal tissue are still visible. In fact, within certain limits this re-agent has very little action upon the animal tissue, and thus at once permits the study of the clarified fungus in relation to its surroundings. It suffices for this purpose that a portion of the tissue containing the black masses should be thoroughly dehydrated in absolute alcohol, then transferred to ether, and subsequently to collodion. The collodion is allowed to concentrate, and the impregnated mass is hardened in methylated chloroform, and cut. The sections are washed in the *Eau de Javelle* until almost all the colour has disappeared from the particles; they are then rinsed in plenty of distilled water and stained with logwood or hæmatoxylin. No matter how old the specimen, and we have examined the oldest in this country, the results are always the same.

The following are the results obtained from an examination of seven specimens of the black variety.[†]

Fig. 4 is a very slightly magnified section of the large mass, fig. 3, obtained from the specimen fig. 2 in University College Museum. It will be seen that it is composed of irregular black masses, which, more especially towards the periphery, have a somewhat branched or radiate disposition. Fig. 6 is portion of one of the dark masses in the preceding figure, seen under the $\frac{1}{12}$ immersion; it shows the indication of a branching network, the branches for the most part running fan-like to the periphery; there is a peculiar oval body in the lower part of the section. Examination of the untreated sections showed the presence of very many of these oval bodies, but some were more round and others more oval in section; during our early experiments with caustic potash their appearance greatly puzzled us. It had occurred to us that they might be sections of vessels, but we could hardly realize how vessels could have become imbedded in the brown amorphous material. Professors MARSHALL WARD and OLIVER likewise

suggested that they might be vessels, and such we consequently found most of them to be. Fig. 10 is a photomicrograph, taken with Obj. BB., ZEISS, of portion of the section fig. 4 which has been treated with *Eau de Javelle* and stained for twenty-four hours in logwood. In it the lighter areas correspond to the slightly stained fungus, whilst the dark network indicates the remains of the animal tissue in which the fungus grew; the bodies conspicuous amongst the hyphal masses are for the most part *vessels*. When fig. 10 is examined with the immersion, the various vegetable and animal elements can be readily made out. Figs. 7 and 8, taken from decolourized unstained glycerine preparations, show that the hyphæ are large, irregularly branched, and septate, and their appearance seems more suggestive of the higher *sprouting fungi* than of any other group with which we are acquainted. The grouping, size, and division of the filaments is, however, subject to considerable variation. The fungus is most frequently met with in the tissue in the form of *tufts*, made up of hyphæ which spread out somewhat fan-like; in a less number of cases the filaments in a tuft radiate regularly from the centre; in a third case (figs. 11 and 12) the periphery is made up of closely-set radiating hyphæ, whilst the centre is occupied by an irregular large-celled pseudo-parenchyma. It was a specimen of this last form that we are inclined to believe Dr. KANTHACK mistook for *Actinomyces*, and upon which he grounded the opinion of the Actinomycotic nature of the white and black varieties of Madura foot. The hyphæ may be very slender, with few septa and branches, a condition which is mostly seen in what appear to be the rapidly growing forms. There may be marked segmentation and branching, the segments may be very large and oval (fig. 8), and in some cases they give rise to a very striking pseudo-parenchyma. Very often one segment is very much larger than the others, and it may be terminal and then resemble somewhat closely a very large spore capsule. In the radiate tufts the hyphæ usually taper towards the periphery. Many of the hyphæ and large cell segments contain granular contents, in spite of the *Eau de Javelle* treatment. The walls of the hyphæ vary very considerably in thickness; sometimes they are as thick as the lumen is wide, and they then cause the filaments in transverse sections to stand out pipe-like; the walls also of the large rounded segments are often irregularly and very greatly thickened; occasionally the walls of the terminal segments of a tuft are thickened, in a club-like manner, fig. 17A. In one case of the disease the majority of the hyphæ were comparatively delicate, fig. 9; in another very large and round or oval (fig. 8). The hyphæ may be very closely packed together into a feltwork, or into a large-celled pseudo-parenchyma, in which the walls of the segments appear to have fused together. On the other hand, the filaments may be widely separated from one another, the intermediate substance being a finely granular ground-glass-like material. This latter substance possesses in the untreated specimens the beautiful golden brown colour which is very characteristic (fig. 6A). The filaments in the centre of the tufts may undergo necrosis. Apart from the colouring material which impregnates the intermediate substance and the walls of the hyphæ, and which can be readily removed

by *Eau de Javelle*, there may exist a much more persistent brown pigmentation of the walls of the filaments (fig. 17A). In no specimen have we seen any indication of the formation of sporangia or of spores.

With regard to the staining reactions of the fungus, we find that logwood, as in the case of the hyphal fungi, generally gives the best results; we further find that the use of the *Eau de Javelle increases* the staining power of the logwood. The filaments often stain unequally; the terminal segments, as a rule, stain more deeply than the others.

The Nature of the Tissue Changes produced by the Growth of the Fungus.

The macroscopic appearances of the foot show that the parasite produces wide-spread hyperplasia as well as tissue destruction. When the relationship of the black particles to the surrounding tissue is more closely studied, it is seen, as previously stated, that the former lie either loosely grouped in the sinuses, or closely surrounded by the tissues and semi-encapsuled. The large body (fig. 3) was encapsuled, but could, nevertheless, be readily enucleated. The readiness with which this mass was removed, as well as its compact nature and appearance on section (fig. 4), led us at first to suppose that it would contain no traces of animal tissue; the clearing away of the yellow colouring material and prolonged staining, however, clearly demonstrated the remains of the tissue in which the fungus grew. The network formed by this tissue is seen in fig. 10, as well as the skeletons of the vessels to which we have already called attention. Fig. 13 is portion of a trabecula of fig. 10, seen under the immersion, and it shows the branching hyphæ dipping into granulation tissue. The drawing (fig. 19) shows the same thing as well as the gradual loss of distinctness of the small round cells; many of the trabeculæ are simply represented by tissue which has undergone coagulation necrosis, and in which, consequently, it is hard to demonstrate any cell elements. Fig. 5, the photograph, and fig. 6A, the drawing, of a radiating tuft of the uncleared fungus, show the massing of small round cells in the immediate vicinity of the fungus. Both in the photograph and in the drawing, the gradual passage of the infiltrated tissue into the homogeneous ground-glass-like and pigmented interstitial substance between the hyphæ of the fungus is very striking. In all preparations of Madura disease, the round celled massing is very obvious, but in some cases the progressive destruction of the infiltrated tissue by the fungus is much more obvious than in other cases. Thus, instead of the fungus being immediately surrounded by small round cell elements, it may be embedded in a wide area of necrotic tissue, in which stains show very little structure; cases like this appear to correspond to rapidly growing forms of the disease. As in the other chronic infective granulomata, so here, in addition to the small round cells, macrocytes and giant cells are abundant. They are found in those cases where the fungus is making the least progress, or, in other words, in those cases in which the resistance of the tissue against the invading parasite is greater. Figs. 17, 21, and 22 show very striking

examples of phagocytosis; the phagocytes are similar to those which one of us found in enormous numbers in the case of *Aspergillus niger*.* Still even more striking are the huge phagocytic giant cells which may very often be found surrounding the smaller masses of the fungus; this appearance is represented in the drawing (fig. 22).

The hyphæ may ramify in the tissues for a considerable distance away from the main mass; this is seen, for instance, in uncleared specimens, where the hyphæ are represented by thick, solid, yellow trabeculæ. Sometimes phagocytes, as in fig. 17, or a large giant cell, indicate the place where a few ramifying filaments may be found upon careful examination. The direction of the ramifications may be determined by that of the connective tissue, trabeculæ, or vessels. We have already directed attention to the concentric vessel-like bodies scattered throughout the large nodule. Many of them enclose several hyphæ, and, from what we have seen in other specimens, it appears that the hyphæ penetrate the vessels, and ramify in them; they serve to guide the hyphæ, and no doubt the same is also effected by the long-coursing connective tissue trabeculæ. In the case of Aspergillar mycosis, previously alluded to, the penetration of a large vessel by the hyphæ was very well marked. It thus appears that the vessels may assist the extension of the fungus to no inconsiderable degree.

Nature and Significance of the Fungus.

CARTER, in his description of the fungus of Madura disease, evidently describes, though with very little detail, a parasite similar to that which we have just described; but he based his conclusions upon specimens which had only *partially* undergone metamorphosis, and in which, therefore, the hyphæ were clear. He concluded from these transition stages that the black masses, as well as the white bodies in the white variety, were of a similar fungoid character. None of our specimens show those transition stages, and, unless we had resorted to the clearing process, it would have been very difficult to demonstrate their fungoid character. It is most probable that the hyphæ are originally clear, and that subsequently pigmentation and metamorphosis occurs. Recognizing the probable fungoid nature of the black masses, CARTER looked upon them as corresponding to the *sclerotia* of the higher fungi. It must be remembered, however, that the hyphæ ramifying throughout the tissues undergo this extraordinary metamorphosis; the process is not limited to a particular portion of the fungus, nor is it connected in any way whatever—at least, in so far as we have been able to judge by the examination of many hundreds of sections—with a process of fructification. As far as we have observed, the interstitial material between the hyphæ is formed, to a slight extent, by the remains of the animal tissue in the immediate vicinity of the hyphæ, and the hyphæ themselves become obscured by an opaque golden-brown pigment: it is not a question of the thickness and browning of the

* R. BOYCE, "On Aspergillar Pneumono-Mycosis." 'Journal of Pathology and Bacteriology,' No. 2, 1892.

walls of the hyphæ, or of their forming a dense pseudo-parenchyma. The phenomenon appears to us to be an end or involution phase, rather than a transition phase. We are aware that, amongst the fungi, more than one *sclerotioid* process is described; but whether anything analogous to the above occurs we are unable, from want of experience, to state. It appears, however, to be a fact that parasitic sclerotia do occur, in which remains of the tissue of the plant host may be seen. DE BARY* mentions that CORDA† points this out in the case of *Peziza sclerotiorum*, but we have not been able to find where he makes the statement in his atlas. DE BARY himself figures *Sclerotinia Fuckeliana*, containing the cell *débris* of its vine-leaf host. In many respects the black masses behave like the sclerotia. Thus, if the sclerotium of of *Claviceps purpurea* is boiled for a short time in caustic potash, and then placed in water, there is the same streaming out of dark brown colouring matter. Both are readily cleared by *Eau de Javelle*. Nitric, hydrochloric, and sulphuric acids cause reddening of the black particles; the same effect is produced, not only upon the sclerotium of *Claviceps*, but also upon those of *Nectria, Peziza sclerotiorum*, and *Rhizomorpha*. As none of these are, however, delicate reactions, we lay no stress upon them. The black particles are very resistant to strong reagents; thus, as has been stated, they may be boiled in concentrated caustic potash for a considerable time, undergoing thereby only a slight change in colour. Sulphuric acid causes their disintegration, but they resist the action of nitric and hydrochloric acids for a considerable time; nitric acid produces some effervescence. Fig. 24 shows a small piece of the section fig. 4 which has been treated with hydrochloric acid, and then with potassic ferrocyanide; there is a marked green coloration of the *peripheral* tufts. We presume the green is due to the combination of a Prussian blue reaction with the natural brown-yellow colour of the fungus, and that it indicates the presence of iron. When the section is first cleared and then tested for the presence of iron, it is again observed that the peripheral tufts acquire a faint *blue* tinge, and, when a portion is highly magnified, numerous blue pigmented bodies, similar to those in fig. 25, are seen; they correspond to small collections of brown pigment which are nearly always to be met with in the disintegrating animal tissue surrounding the hyphæ; the iron in the colouring matter is probably for the most part of vascular origin. Thus a microchemical examination of the dark masses furnishes good evidence of the presence of iron, and this exactly accords with the chemical analysis. Chemical analyses have been made both by BRISTOWE and by LEWIS and CUNNINGHAM, and have revealed the presence of iron. BRISTOWE states that a small quantity of ash was left after combustion, and that it contained a little oxide of iron, but much less than a similar quantity of altered blood would have contained after combustion. According to LEWIS and CUNNINGHAM, the ash is of a red colour, owing to the presence of oxide of iron, and this fact points to an origin identical with the other pigmentary

* DE BARY, 'Comparative Morphology and Biology of the Fungi.' Oxford, 1887.
† CORDA, 'Icones Fungorum.'

deposits in animal tissues. Spectroscopic examination of the colouring matter of the brown masses does not any way favour a blood origin. We have ourselves incinerated thick pieces of the large black mass. The dried particles burn with a luminous flame. There is a smell of "burnt feathers." Heated on the platinum capsule, there is no sputtering whatever, and a residue is left white in the centre but brown at the periphery; the latter contains iron, giving the Prussian blue test, so that this peripheral distribution of the iron quite accords with the above micro-chemical observations. The iron is probably derived from the tissues, and not from the fungus. Whether the yellow pigmentary substance is of a resinous nature, and similar to that produced by certain species of fungi, it would be difficult to say. The dried particle burns readily; it is only very slightly cleared by boiling in carbon bisulphide, benzole, xylol, chloroform, and ether, and the special cupric acetate test for resinous substances fails, there is also no reaction with ferric chloride.

It is clear that, in the "sclerotioid" phase of the fungus, we have to deal with a process taking place in the living tissues which appears to be quite unique, and all the evidence is in favour of the view that the yellow colouring matter is furnished by the fungus.

One specimen of the black variety, from which figs. 11, 12, and 17 are made, shows unmistakeably that the tissue is offering considerable resistance to the invasion of the parasite, and that in consequence the latter is altered in appearance. The tufts are radiate, but, instead of the ends of the hyphæ being loosely arranged, they are grouped into a dense palisade, as seen in fig. 17A; some are thickened and club-like, and they are invested by either phagocytes, fig. 17, or giant cells, fig. 22. The interior of the tuft is occupied by an irregular large cell pseudo-parenchyma, which is slightly pigmented, and between this and the above-mentioned palisade, the hyphæ much reduced in size form a deeply pigmented dense zone, figs. 12 and 17A. This type is interesting to compare with those specimens in which both the tufts are very numerous, and the hyphæ run in the one direction, whilst at the same time the destruction of the tissue is great.

As regards the relationship of this fungus to Madura disease, we think that the anatomical evidence brought forward shows that it is pathogenic. The fungus is scattered in large quantities throughout the tissues, and there appear around it areas of necrosis, of granulation tissue, of phagocytes and of phagocytic giant cells. The process *spreads*, and fresh areas of the foot, of the leg, or even of the thigh become invaded by the black particles. But it is very extraordinary that in our six specimens we have only met with one in which the peculiar pigmentary metamor-phosis was not marked. Owing to the rapid metamorphosis it seems to us that the cultivation of the fungus from the particles will be difficult. Two doubtful cases have been recorded by CARTER, and the other by LEWIS and CUNNINGHAM, of the occurrence of the black and white particles in the same specimen. We likewise possess in our college a specimen in which both black and white particles were

found; but, whether owing to the bad preservation or not, we have always failed in this specimen to demonstrate the tissue changes which could be attributable to the growth of the fungus; there appears in this specimen no relationship between the black fungus and the tissue, and it is very possible that the black particles may have fallen into the cavities of the foot, from being placed in the same vessel along with specimens of the black. On the other hand, the presence in the same foot of two distinct parasites, or of one parasite succeeding the other, is far from being an impossibility.

The case of BASSINI, mentioned at the commencement of this paper, is interesting in connection with the pathogenicity of this disease. In this case the patient pricked his foot in an Ox stall. The wound healed, but a tumour gradually formed which broke upon the surface, and, between the seventh and eighth month after the injury, prevented walking. The tumour, which had reached the size of a pomegranate, was removed; it was found to be pervaded by dark brown or black particles varying in size from a pin's head to a hazel nut, and there were numerous fistulæ from which the black particles protruded. As previously mentioned, treatment with caustic potash revealed a septate mycelial fungus. BASSINI did not succeed in cultivating it.

The White Variety of Madura Disease.

Of this variety we have examined sixteen cases, and we have found that in those specimens where the characteristic roe-like bodies are present the structure is uniform. The appearance of a section of a particle under the low power, is well seen in fig. 14. It is extremely characteristic, and it appears to us difficult to conceive how it could have been confounded with the black variety. The particle consists of an aggregation of deeply staining "reniform" bodies and of a radiate external zone. In this section the particle is seen to be surrounded by granulation tissue, but it may be free in large numbers in the sinuses and be discharged with the pus as the fish-roe bodies. Usually about the size of a pin's head they may yet be often found of the size of a pea; they are most frequently nodular upon the surface. They are soft and friable, and possess a very light brown or, as it is termed, "ochroid tint," but they are never yellow, as in *Actinomyces*.

A considerable number of the observers who have examined these bodies have failed to find any vegetable structure, whilst in the case of those who have recognised a fungus, the descriptions have, we venture to think, been rendered very misleading, owing to confusing the two varieties.

The particles are, to a great extent, concretions of caseous and probably phosphatic materials upon a nucleus which possesses traces of a fungus. To examine the fungus it is, therefore, necessary to remove as much as possible the foreign materials. To do this we treat either the free particles or the sections of the particles in the tissue with the various fat solvents. Thus the particles are *heated* in absolute alcohol, ether,

carbon bisulphide, chloroform, xylol, or benzole, for from a few minutes to half an hour or longer.   Particles treated in this manner are clearer and take up the logwood stain much more readily than untreated specimens ; yet, notwithstanding, their bulk cannot be said to be much diminished ; a quite similar result may be observed in the case of caseous material.   Definite acicular crystals are often met with after treatment with the above-mentioned fat solvents, so that they are probably not fatty.   After these reagents we very frequently employ fuming hydrochloric acid ; this renders the outlines of the fungus sharp, but there is only a slight solvent action.   In contrast to the black variety, strong caustic potash, or *Eau de Javelle*, cannot be used, as those reagents soon cause the complete destruction of the particles.   This shows that the vegetable structure cannot be very resisting.   For embedding sections we always employ the paraffin ether method ; celloidin gives most misleading results, as may be readily seen by comparing side by side sections prepared in the two ways.

The dark kidney-like masses seen in fig. 14 are most probably fungoid.   They stain very deeply with most aniline and logwood stains, but it is exceedingly difficult, notwithstanding, to make out a definite vegetable structure.   They appear for the most part to be granular, the granules being small and very densely packed.   We have seen, however, in two specimens, unmistakeable evidence of a very delicate branched hyphal network.   One of these cases has been recently described by Dr. Hewlett,[*] and, from the appearance, he concluded that it was a fungus similar to *Actinomyces*.   The other case was one examined by Dr. Kanthack.   We have ourselves examined an early and well preserved foot which was sent to us direct from Bombay.   There are no particles visible to the naked eye, but the sections show great leucocytic massing, and the commencement of the formation of minute abscesses and sinuses.   Here and there in the leucocytic centres, small granular reniform or annular masses may be seen which stain only slightly with gentian violet ; here, occasionally, however, very short and extremely delicate deeply stained filaments may be seen in the apparently granular masses but there is no radiation, nor are there clubs to suggest a relationship with *Actinomyces*.   Fig. 15 shows an appearance also very commonly met with in the particles, namely, the formation of a *deeply staining* irregular fringe around the reniform bodies.   Examined with the high power, the fringe may be seen to consist of stunted, rather thick hyphæ ; sometimes there is a very large number of the stunted projections, and double staining with Hoffmann's green and eosine may give very striking pictures, the fringe staining green, and the reniform masses a reddish-purple.   The walls of these dwarfed club-like processes may be greatly thickened, and they closely resemble many of the clubs to be seen in the ray fungus.   We have not in our specimens, however, succeeded in tracing the hyphal processes into the central granular masses.   Exceptionally the clubs are long.   A third characteristic of the white particle is the radiate zone which

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* Hewlett, 'Lancet,' 1892.   Dr. Hewlett has very kindly shown us his preparations and given us material, and we agree with his description.

surrounds the reniform masses. The appearance of this radiation is extremely well shown in the very distinct high power photo., fig. 16. Exceedingly delicate straight brush-like processes radiate out for a considerable distance from the central deeply staining mass, whilst between the radii are numerous compressed leucocytes. In a few cases the slender radii are thicker and more club-like, in others they are represented by thick radiate refractive masses in which no structure can be made out.

Such then are the leading features which we believe can be seen in the roe-like particles; some of them favour a relationship with *Actinomyces*, but from others we cannot in our present state of knowledge draw any conclusions. The deep radiation is a most remarkable phenomenon, and one which has greatly struck both CARTER and LEWIS and CUNNINGHAM; these observers looked upon them as consisting mostly of fat crystals, and although we also think that fat crystals may greatly increase the radiate appearance, yet we have found the radiate zone, as in figs. 14 and 16, after prolonged subjection of the sections to the fat solvents mentioned above. Coupled with these curious facts we have found others equally hard to interpret. Thus, in addition to the opaque ochroid roe-like bodies, we have met with others, transparent and gelatinous, which, when pressed under a cover slip, exhibited a radiate structureless and refractive peripheral zone; yet, from microchemical testing, we could draw no conclusions as to their nature. Similarly, when we read the records of CARTER and LEWIS and CUNNINGHAM, we find that they record cases in which the specimens contained innumerable cayenne pepper-like granules.

Comment upon these various anomalies would be out of place, and therefore, in spite of the fact that although we have examined comparatively a very large number of specimens, we think it would be rash to state that the white particles are always Actinomycotic, and may not in certain cases represent some other parasite.

Conclusion.

The object of this paper has been to show that in the Madura foot disease the white and black particles are of different nature, and to set forth the means whereby these differences can be displayed.

ADDENDUM.

Since this paper was written we have had the opportunity of examining a very large number of specimens of Madura disease, including fifteen examples of the black variety. For these we have to thank Professors GREENFIELD, of Edinburgh, and HAMILTON, of Aberdeen, and Dr. ROLLESTON, of St. George's Hospital, but especially Brigade-Surgeon Lieutenant-Colonel KEITH and Dr. BOCCARO, of Hyderabad, Sind. Dr. BOCCARO sent to us the MS. of an analysis of *one hundred* cases of Madura disease treated in the Hyderabad Hospital, and portion of this was published in the 'Lancet,'

September, 1893.   *The vast majority of these cases appear to belong to the black variety*; and, with one or two exceptions, the specimens sent to us from Hyderabad, Sind, belong to the black variety.   This points to a local distribution for both the black and the white variety.   We may add, also, that we find considerable differences between the gross anatomical changes met with in the black and white varieties respectively.   Dr. Boccaro finds, in 17 of the 100 cases analysed by him, evidence of the pricks of an Acacia thorn (the "Babul"); in many of these cases the thorn was found.   He also records a case of mycetoma between the vertebral column and the scapula.   Dr. Keith has kindly made for us very careful inquiries for the presence of Madura disease in cattle, and has been informed by the Veterinary Surgeon of its occurrence; in one case in the foot of a Camel.   Dr. Keith will, however, not pronounce a decided opinion till he has had an opportunity of examining a specimen for himself.

Drs. Keith and Boccaro have also inoculated several glycerine-agar tubes for us, but we have not succeeded in cultivating the black particles.   We are, however, expecting more, and, one of us having left for India, we hope shortly to be able to transfer our test-tube experiments to the animal.—December 11th, 1893.

DESCRIPTION OF PLATES.

PLATE 1.

Fig. 1. Specimen of the white variety, from the Museum of Guy's Hospital.   There is enormous overgrowth of the foot, and the toes are almost completely buried.

Fig. 2. Section of a foot in University College Museum, of the black variety.   There are extensive cavities which were filled with black masses.

Fig. 3. A black nodule, natural size, from the preceding specimen.

Fig. 4. A section through the preceding, magnified a few diameters.

Fig. 5. Section of a small nodule of the fungus *in situ* in the tissue, in an early stage.   In the fan-shaped semicircular patch a distinct radiation is observed; in two other patches, cut tangentially, the hollow lumina of the hyphæ are seen.   Zeiss, obj. D.

Fig. 6. Portion of a small tuft in fig. 4.   An indication of branching hyphæ is seen. Zeiss, obj. $\frac{1}{12}$; no eyepiece.

Fig. 7. Portion of same, after clearing.   Zeiss, obj. $\frac{1}{12}$; no eyepiece.

PLATE 2.

Fig. 8. Another portion of same, after clearing, and equally magnified.   At the periphery the ends of the hyphæ tend to form a palisade.

Fig. 9. Clarified hyphæ from another specimen. ZEISS, obj. $\frac{1}{15}$.

Fig. 10. Portion of fig. 4, clarified and stained with logwood. The lighter patches are occupied by the fungus. The darker stained portions represent granulation and vessels. Low power.

Fig. 11. Fungal tufts from a case of the black variety in St. Bartholomew's Hospital. Obj. 1 in.

Fig. 12. The same, under the $\frac{1}{4}$ in. obj. It shows the formation of a palisade at the periphery.

Fig. 13. Portion of fig. 10, showing the termination of a few hyphæ in the neorotic granulation tissue.

PLATE 3.

Fig. 6A. A coloured drawing of fig. 5, stained with logwood. a, b, particles, natural colour ; c, granulation tissue.

Fig. 17A. Portion of the periphery of fig. 12. a, internal hyphal network and pseudo-parenchyma ; b, denser pigmented zone ; c, the ends of the hyphæ, forming the palisade and clubs. Obj. $\frac{1}{15}$.

Fig. 19. Coloured sketch of another portion of fig. 13.

Fig. 21. Examples of phagocytosis, from the specimen fig. 12. Obj. $\frac{1}{15}$.

Fig. 22. A tuft from the same specimen, surrounded by giant cells.

Fig. 24. A small portion of fig. 4, treated with HCl and potassic ferrocyanide. The peripheral tufts give a greenish reaction. Slightly magnified.

Fig. 25. Portion of same after clearing, and similar treatment. There are numerous collections of dark brown granules in the neorotic tissue, which give the Prussian-blue reaction.

PLATE 4.

Fig. 14. White variety of Mycetoma. Section through a roe-like body *in situ*. Obj. 1 in.

Fig. 15. Another specimen, in which the reniform bodies are not stained, as in the preceding specimen. The periphery only is stained. Obj. 1 in.

Fig. 16. Portion of the radiate zone in fig. 14. Note the compressed cells. REICHART, $\frac{1}{12}$ apochromat. + comp. ocular.

Fig. 17. Examples of large phagocytes, adhering to hyphæ in specimen fig. 12. Obj. $\frac{1}{15}$.

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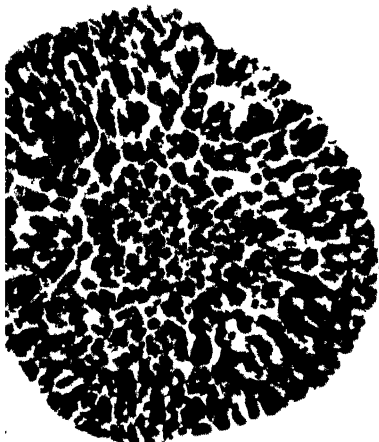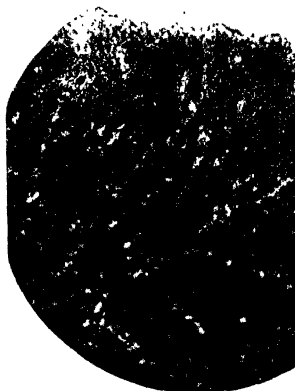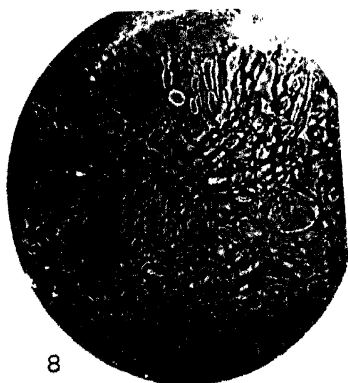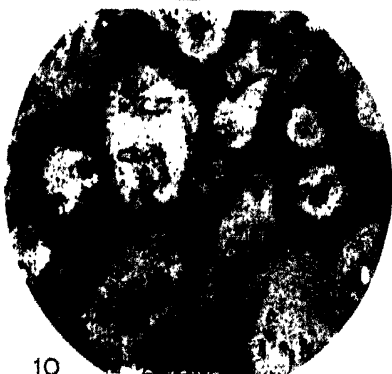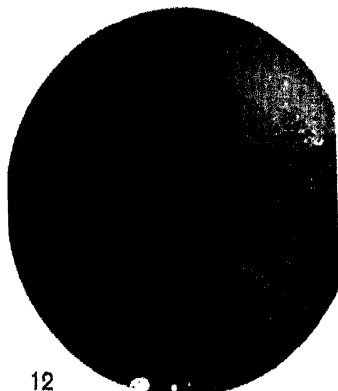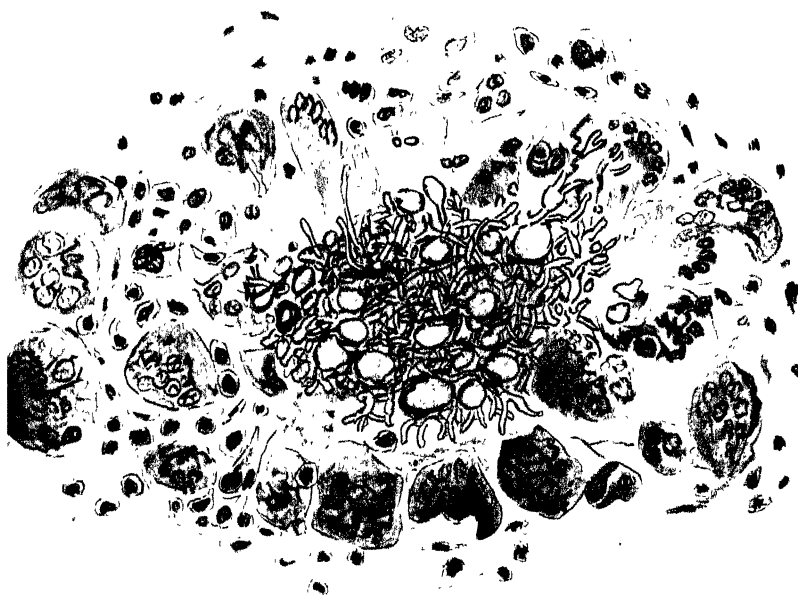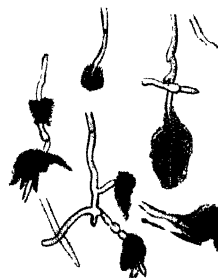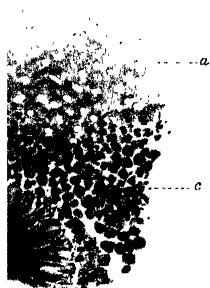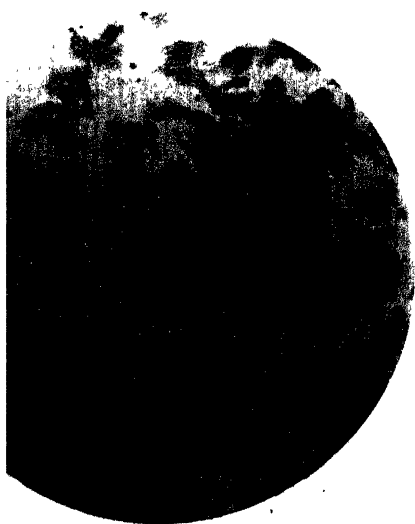
II. *On* Megaladapis madagascariensis, *an Extinct Gigantic Lemuroid from Madagascar; with Remarks on the Associated Fauna, and on its Geological Age.*

By C. I. FORSYTH MAJOR.

Communicated by HENRY WOODWARD, *LL.D., F.R.S., V.P.G.S.*

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[PLATES 5–7.]

CONTENTS.

I. INTRODUCTION.

MORE than forty years ago, the scientific world was startled by the discovery made in Madagascar, and announced by ISIDORE GEOFFROY SAINT-HILAIRE to the French Academy of Science,[*] of the eggs and bones of a gigantic bird, to which the name of *Aepyornis maximus* was assigned.

Though these were not the first proofs of an extinct Vertebrate Fauna in that island,[†] the discovery of eggs, said to have eight times the volume of those of *Struthio*, was of a nature to excite a wider interest, and gave rise to many speculations.

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[*] ISID. GEOFFROY SAINT-HILAIRE, " Notice sur des ossements et des œufs trouvés à Madagascar dans des alluvions modernes et provenant d'un oiseau gigantesque." ' Comptes Rend. Acad. Sc.' du 27 janv. 1851, p. 101–107. *Cf.* 'Ann. des Sc. Nat.,' 3 série, " Zoologie," vol. 14, Paris 1850, p. 206–213.

[†] See ' Proc. of the Geol. Soc. of London,' vol. 1, 1833, No. 31, p. 479. P. GERVAIS, in 'Dictionn. des Sc. Natur.,' Supplément. 1841, p. 254 : " L'Autruche d'Afrique n'a donné lieu à aucun travail nouveau que nous connaissions. Nous dirons seulement, comme pouvant se rapporter à un oiseau du groupe de ces animaux, que nous avons vu, il y a plusieurs années, des débris d'œufs qui *paraissent avoir dû être du volume de ceux des Autruches*, et que M. GOUDOT avait trouvés dans l'île de Madagascar, mais sans avoir à leur sujet des renseignements positifs."

Since that time, occasional, though not numerous discoveries, to which I shall have incidentally to refer hereafter, have somewhat increased our knowledge of the so-called sub-fossil Vertebrate Fauna of Madagascar.

Recently, the British Museum acquired remains of Vertebrata, discovered in a fossil condition in various parts of the island. One of the most remarkable is a Mammalian skull of strange aspect, which forms the subject of the present communication, and for which the name of *Megaladapis madagascariensis* is proposed.

I am indebted to Dr. HENRY WOODWARD, Keeper of the Geological Department, British Museum (Natural History), for the permission to describe it.

The specimen was sent from Madagascar by Mr. J. T. LAST, a collector of the Hon. WALTER ROTHSCHILD, and is said to have been found in a marsh at Amboulisatra, on the south-west coast of Madagascar, together with scanty remains of several other Vertebrata, to be referred to hereafter.

II. DESCRIPTION OF THE SKULL.

The base of the skull being wanting in almost the whole length of the floor of the brain-case, the exceedingly short and low brain-cavity is exhibited. The bullae osseae are broken away, and the foremost facial portion is also wanting, so that nothing can be said about the anterior parts of the dentition, the canines and incisors. The right and left ramus of a mandibula, somewhat incomplete in their anterior and posterior portions, were found together with the cranium, and, apparently, belong to the same specimen.

The length of the whole skull may be approximately calculated at 250 millims., and supposes an animal of from three to four times the size of a common Cat.

By the worn condition of the teeth, the obliteration of most of the sutures of the very thick bones, and the strongly developed crests, it is shown that the individual was much aged.

One of the most striking features of this cranium consists in the enormous lateral development of the interorbital region of the frontals, so as to form a broad and elongate roof over the orbits, which protrude in a tubular form outwards and forwards, and are encircled by a complete bony ring, with thick rounded margins. Compared to the size of the skull, the diameter of the orbits is small, suggesting diurnal habits. Beneath the postorbital bar, the orbits open freely into the temporal fossa.

The foramina lacrymalia are situated externally to the margin of the orbits. The lacrymal bones, as far as can be ascertained, are of small dimensions. On account of the partial obliteration of their sutures, the limits of the nasals cannot be quite exactly determined; these bones are not flattened, but convex from side to side, and separated by a groove from the maxillaries; they extend backwards with attenuated dimensions as far as the middle of the orbits. The facial portion is elongate and the maxillaries high.

The middle line of the frontals is feebly convex, forming an anterior continuation of the sagittal crest ; from the convex middle line the frontals slope down gently on both sides, the roof of the orbits being again gently raised, so that the frontal region between these last and the middle line is somewhat depressed.   Behind the orbits the frontals are much narrowed, and stretch backwards on the superior surface as well as laterally, the almost obliterated coronal suture being distant 31 millims. from the posterior margin of the orbits.   Corresponding to the suture we find a very slight lateral constriction of the bone, behind which the parietals extend, with a breadth about equal to that of the frontals, and separated in the middle by a sagittal crest, which is less remarkable for its height than for its extraordinary thickness and flat-ness.   Backwards from the orbits the crests divide into two portions, which continue anteriorly in a curved line, and come to an end on the roofs of the orbits.

Behind the molar series the maxillaries stretch upwards and backwards to an unusually great extent, and in this region are greatly inflated by aërial sinuses, which extend forward in the facial region above the molar series, and communicate behind with the frontal.

The occipital region is truncated, and divided by a median strong and sharp crest into two hollows.   It is considerably narrowed, as is the whole of the skull, backwards from the orbits.   The vertical direction of the occipital condyles, as well as the narrow but high *foramen magnum*, appears to be in connection with this lateral compression of the skull.

The zygomatic arch is high and but moderately projecting outwards, thus contri-buting to the general narrowed appearance of the posterior portion of the skull.

The cranio-facial angle is extremely obtuse, as in most lower Mammals ; but whilst in these last the angle is open downwards, it would seem to be open upwards in *Megaladapis*, in consequence of both the facial and the cranial portion being some-what bent upwards, the first anteriorly, the second posteriorly.   Corresponding with this upward bending of the facial portion, the palate is convex in an antero-posterior direction, the convexity looking downwards.

Other characters of the cranium will be mentioned and discussed in the following paragraphs.

Mandibula.—The symphysial suture is completely obliterated.   The inferior out-line of the bone forms an almost straight line, with a slight downward bending anteriorly.   The upper alveolar margin is far from parallel with the lower one ; when this last is held horizontally it appears that the upper margin, and accordingly the series of the teeth, form an arched line, curving upwards anteriorly.   This structure corresponds to a similar upward curving of the superior dental series, and is, of course, in relation with the upward bending of the facial portion.   As a consequence, the anterior portion of the horizontal mandibular ramus is considerably higher than is the hinder (see measurements).   The lower third of the horizontal ramus is attenuated, as compared to the upper two-thirds, there being a longitudinal groove on

the inner inferior part of the bone, more deepened in the posterior moiety, more shallow anteriorly.

The ascending ramus is considerably attenuated, and, as far as can be judged from the part preserved (the posterior and superior portion being broken), it was notably elongate antero-posteriorly.

In the right mandibular ramus four teeth are preserved, viz., the three molar and the posterior premolar; anterior to this last, the alveolus of a second, double-rooted premolar is visible.

The cusps of the inferior *molars* are obliquely disposed, the two outer ones alternating with the three inner ones, the anterior of which is very feebly developed. The two outer cusps are divided by a *sulcus*, which scarcely reaches the centre of the molar. The third molar is provided with a talon, consisting of a single strong cusp. An outer basal cingulum exists on the outer side of the three molars. The premolar has a single outer cusp, from the anterior and posterior side of which depart two crescents, which terminate on the interior side in a moderately developed anterior and posterior cusp; while between them a central interior cusp, somewhat stronger, and almost completely coalesced with the single outer cusp, is seen.

The *superior molars* (three in number) are of a simple tritubercular type, there being two external and one internal cusp. This last is deeply divided from the postero-external cusp; but from its anterior side a crest extends towards the outer part of the antero-external cusp. The superior premolars were three in number, as far as can be judged; two are in place, and anteriorly to them the alveoli of a two-fanged tooth are visible on the right side. The two premolars have each one outer and one inner cusp; and, like the molar, an inner and two outer roots. Anterior to the alveoli is a diastema, which on the right side is preserved to the extent of 10 millims.; further on the bone is broken.

MEASUREMENTS of the Teeth.

From the posterior margin of m_3 sup. to the anterior margin of the alveolus of p_3, 74·5 millims.

m_3 sup., greatest length, 17·8 millims.; greatest breadth, 14·6 millims.
m_2 sup.	,,	17	,,	,.	15·8 ,,
m_1 sup.	,,	13·5	,,	,,	12·7 ,,
p_1 sup.	,,	10	,,	,,	9·7 ,,
p_2 sup.	,,	10·3	,,	,,	8·5 ,,

Breadth of the outer margins of the alveoli of p_3 sup.: 9·8 millims.

FRAGMENT of Right Maxillary of a Second Specimen.

greatest length, 18·5 millims.

18 ,, ; greatest breadth (at the base) 15·5 millims.

<div align="center">INFERIOR Molars.</div>

Length of m_3—m_1 . . . . 52·5 millims.

,, p_1 10   ,,

,, m_1 13   ,,

,, m_2 . . . . 15·5 ,,

,, m_3 . . . . 23·5 ,,

<div align="center">MANDIBULA.</div>

Height of the horizontal ramus beneath the anterior margin of p_1, 55 millims.

,,   ,,   ,,   ,,   posterior   ,, m_3, 40   ,,

<div align="center">DIMENSIONS of the Skull.</div>

From under margin of *foramen magnum* to a line uniting the anterior
    margins of the alveoli of p_3 195   millims.

Greatest breadth (between the middle of the orbits) 111   ,,

Approximate length of the frontalia in the middle line . . . . . 47·5 ,,

Length of the undivided sagittal crest . . . . 72·3 ,,

Foramen magnum to posterior part of palate . . . . 130·3 ,,

Greatest breadth between the zygomatic arches 105   ,,

Approximate greatest breadth of nasalia 34   ,,

Breadth of frontalia (narrowest part) at sutura coronalis . . . . . 37·5 ,,

Breadth of palate between the postero-external angles of the last molars 55·8 ,,

    ,,   ,,   ,,   alveoli of the last molars 48   ,,

Approximate length of brain cavity (from under margin of the
    foramen magnum to the boundary between cerebral and
    olfactory fossa) . . . . 71·5 ,,

<div align="center">III. AFFINITIES.</div>

A *superficial* examination of the skull of *Megaladapis* will certainly not suggest
its classification amongst the *Lemuroidea*, from which it seems, *à priori*, precluded by
its comparatively enormous size. Besides, we associate in our mind with the idea of
a Lemurid cranium large orbits, approaching closely in the middle line and directed
straight forward ; a rounded and relatively large cranium proper ; the covering of the
brain-case due chiefly to the parietals, the frontals extending laterally backwards but
slightly from the orbits, and the squamosals not rising high up vertically to meet the
parietals ; almost general absence of crests, which, when present (*Tarsius, Galago,
Lepidolemur*), are but feebly developed ; a slender zygomatic arch ; long persistence

<div align="center">D 2</div>

of the sutures. In all these characters the skull before us, as described in the preceding paragraph, is the exact reverse of what, judging from those Lemurids which are most familiar to us, we are inclined to consider as typical of the Lemurid skull ; whereas, on the other hand, some features in the " physiognomy" of our fossil cranium seem to point to quite other directions. These we have first to consider.

The high and moderately outward-curved zygomatic arch ; the backward prolongation of a rather narrow frontal region, which is continued with a slight constriction into an equally narrow parietal region ; the squamosals extending rather high upwards to meet the parietals, and thus partaking to a considerable extent in the covering of the brain-case ; the strongly developed sagittal crest, concealing somewhat the depressed form of the skull ;—all these are features which at once call to mind the cranium of a Marsupial, and especially *Phascolarctos*. Added to this, the narrow, short, and low brain-case, indicated externally by some of the characters mentioned (and for which the only analogy is found amongst Marsupialia and Insectivora, *e.g.*, the *Centetidæ*, considered to be amongst the most lowly organized of all placental Mammalia), would seem to afford a strong aprioristic assumption against the association of *Megaladapis* with any of the *Lemuroidea*.

Besides, there are other features in the skull of *Megaladapis* which form some approach towards the South American Howlers (*Mycetes*). In *Megaladapis*, as well as in Marsupialia (*e.g.*, *Thylacinus*, *Phascolarctos*), the cranial portion of the skull is situated at a considerably higher level than the facial portion when the palate is horizontal ; this, in the Marsupials, is exclusively owing to the small dimensions of the brain cavity. In *Mycetes*, although this genus is provided with a much more voluminous brain-case than the Marsupials and *Megaladapis*, the back part of the skull is still more elevated above the facial portion, and especially above its posterior moiety. This conformation is the result of the upward bending of the posterior part of the cranial portion, and partly of an equal upward bending of the anterior part of the snout, which result in the well-known elongate pyramidal shape of the *Mycetes* skull. At the same time the palate is arched downwards antero-posteriorly. It has already been pointed out that *Megaladapis* presents similar characters. However, the bending up of the anterior facial portion, and, as a consequence, the downward convexity of the palate, are more strongly developed in *Mycetes*. In relation with this conformation is the considerable hollowing at the roots of the nasals in *Mycetes*, whereas in *Megaladapis*, although the upper profile of its cranium slopes down abruptly from behind forwards, as in the former, the line formed by the profile is almost a straight one, there being but a very slight hollowing anterior to the orbits. The occiput is truncated in both.

The curious shape of the Howler's skull is partly related to the peculiar modification of the hyoid bone, with which is in relation, too, the considerable development of the mandibular rami. As far as can be judged from the somewhat incomplete condition of the mandible of *Megaladapis*, the ascending portion was unusually elongate antero-

posteriorly, though vertically it extends less than in *Mycetes*. There exists, therefore, a strong assumption that, as in *Mycetes*, the Malagassy fossil was provided with vocal organs of unusual size. This specialization, however, does not in the least imply a nearer relationship with *Mycetes*, for in numerous other characters of its skull *Megaladapis* departs from the South American genus.

I have placed in the strongest possible light the reasons which might be advanced against ranging *Megaladapis* amongst the Lemuroidea ; we have now to review and weigh them, one after the other. As to the shape and extension of the interorbital frontal region, *Tarsius* represents one extreme, the orbits coming almost in contact in the middle line. But, proceeding from this genus, we meet with all possible gradations in this respect, the stages being *Nycticebus*, *Perodicticus*, *Lepidolemur*, *Hapalemur*, *Lemur*, and lastly the *Indrisinæ*, in which the interorbital region is considerably extended in a lateral direction, and most so in *Avahis*, though still much less than in *Megaladapis*, which presents the opposite extreme of *Tarsius*. The general depression of the interorbital region of *Megaladapis* is to be seen as well in the Tertiary Lemuroid *Adapis*; amongst existing forms in *Lepidolemur*, and to a somewhat lesser degree in *Hapalemur*.

Neither is the anterior direction of the orbits a constant character of Lemuroids ; within the subfamilies *Galaginæ* (*Chirogale*) and *Lemurinæ* (*Hapalemur, Lepidolemur*) we meet with instances in which the orbits are more directed outwards than in others. *Adapis* presents likewise variations in this respect. In the typical skull of the smaller form, *Adapis parisiensis*, the orbits show a decidedly anterior direction ; in *A. magnus*, as well as in a skull united by FILHOL with the smaller species,[*] the direction is almost the same as that seen in *Megaladapis*, although they are far from possessing the peripheric position and the tubular form of the Malagassy fossil.

The rounded, vaulted cranial portion is characteristic for all existing Lemurids, with some variation (*e.g., Chirogale Milii*, in which the cranial portion is somewhat more flattened than in *Lemur*), as opposed to *Megaladapis*.

In *Adapis*, by the depression of this part, coupled with the enormous, though sharp, sagittal crest, a strong approach is made to *Megaladapis*. The brain-case of this last is comparatively much shorter and narrower than in the first.

A very elongate facial cranium is presented by the genus *Lemur*. As in many other natural groups, we meet with a great variety amongst Lemurids in this respect. *Lemur* on one side, the *Indrisinæ*, with a very short snout (*e.g., Avahis*), on the other, are the extremes. *Adapis*, though possessing four premolars, has a much shorter facial portion than *Lemur*.

The zygomatic arch, generally slender in the *Lemuridæ*, acquires greater strength and is higher in the *Indrisinæ*. Still closer approach is made to *Megaladapis* by *Adapis*, in which, besides, the squamosal rises still higher than in the former. In

Megaladapis the vertical extension of the parietal is the double of that of the squamosal; in *Lemur* the first is four times higher than the last; whereas in *Adapis*, on the contrary, the vertical extent of the squamosal, near the occipital crest, is scarcely less than that of the parietal, and even exceeds it, if the sagittal crest be disregarded. In *Lemur*, as well as in most existing Lemurids, the great disproportion in the respective heights of the parietal and squamosal, is in relation with the greater volume of the brain-case; this does not, however, explain the vertical development of the squamosal in *Megaladapis*, and the still greater in *Adapis*, in which last a nearer approach to the Marsupialia is given than by *Megaladapis*. How little classificatory value can be attributed to this structure is sufficiently illustrated by the fact that we find very high parietals amongst Ungulates and Rodents as well. This same remark applies to the vertical extension of the zygomatic arch.

In a skull of *Adapis parisiensis* (Br. Mus.) the occipital condyles approach to the vertical position exhibited in *Megaladapis*. They are perfectly vertical, as in this last, in a skull of *Galago* from the Kilima-Njaro (Br. Mus., 92, 10, 18, 10) and in *Perodicticus potto*.

In other existing Lemurids, as *Lemur*, *Hapalemur*, *Propithecus*, in which the horizontal diameter of the foramen magnum is equal or superior to its vertical diameter, the anterior portions of the condyles are directed inwards, the posterior portions externally.

In some essential structures *Megaladapis* agrees with the *Lemuridæ*. Such are the orbits, which form a complete bony ring, and communicate freely with the temporal fossa beneath the postorbital bar; the lacrymal foramen, which is situated externally to the margin of the orbit; the horizontal mandibular ramus, which is higher in proximity to the symphysis, than further back; in the shape of the molars, *Megaladapis* is closely related to *Lepidolemur*, and more so to the smaller forms included in the genera *Microcebus* and *Chirogale*. The inferior molars, also, are of the same type as *Adapis*, with the difference that the anterior portion of the *Adapis* molars is less atrophied, the anterior transverse valley being better developed. Further, in *Adapis*, the last molar has only two internal cusps.

IV. PRIMITIVE ANCESTOR OR DEGENERATE DESCENDANT?

The question next arises as to whether, in the characters of its skull, *Megaladapis* must be regarded as a generalized or specialized member of the Lemuroidea. In other words, is *Megaladapis* a primitive ancestor or a degenerate descendant? An answer to the question has already been hinted; but it seems advisable to attempt a fuller discussion, dealing with the dentition and the cranium separately.

With regard to the dentition, the majority of palæontologists would doubtless regard the molars as primitive; the purely tritubercular type of the upper molars and the corresponding simplicity of the lower molars being usually considered to indicate a

generalized condition. I have, however, recently discussed this matter at length,* and it thus suffices on the present occasion merely to point out how *Megaladapis* confirms the arguments I have adduced against the validity of the "tritubercular theory." No Mammal fauna is more appropriate than that of Madagascar for consideration in reference to the problem, and *Megaladapis* is an important discovery in this connection.

In Madagascar, the so-called tritubercular type of molar is found in families of three distinct orders, namely, in the Viverridæ, Centetidæ, and Lemuridæ.

Fig. 1 nat. size   *Fig. 2 × 4*   *Fig. 3 × 4*   *Fig. 4 × 4*   *Fig. 5 × 7*
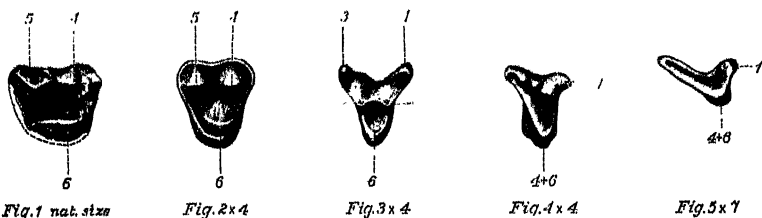
Fig. 1. *Megaladapis madagascariensis*, m_2, sup. dext.—Fig. 2. *Chirogale Milii*, m_2, sup. dext.—Fig. 3. *Eupleres Goudoti*, m_1, sup. dext.—Fig. 4. *Centetes ecaudatus*, m_1, sup. dext.—Fig. 5. *Hemicentetes madagascariensis*, m_2, sup. dext.—The method of numbering the homologous cusps is the same as that adopted by WINGE ('Vidensk. Meddel. Naturh. Foren. i. Kjoebenhavn,' 1882.)

On the widely accepted theory, it would therefore be necessary to assume some close relationship between these three families, and also between them and *Megaladapis*. I feel sure that not one of the adherents of trituberculism will venture to uphold such a view, although it would be consistent with the theory. But it will be asserted that they must rather be regarded merely as lowly organized representatives of their respective orders.

This assertion is disposed of by the fact that each family comprises some form showing a tendency to further reduction of the cusps of the molars, viz., the Carnivore *Eupleres*, the Insectivore *Hemicentetes*, and the Lemuroids *Chirogale Milii* and *Chiromys*.

These same genera, as well as *Megaladapis*, exhibit, moreover, evidence of retrogressive evolution in other characters. Hence it is reasonable to conclude that the tritubercular condition of the molars is the result of similar evolution, and by no means a primitive condition.

We may even go further, and combat the belief that the tritubercular type of molar has had a common origin. If we compare, for instance, the lower molars of *Centetes* and *Megaladapis* (or *Chirogale*), we observe that in the first, the posterior part of the

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* "On some Miocene Squirrels, with Remarks on the Dentition and Classification of the Sciurinæ" ('Proc. Zool. Soc.,' London, February 28, 1893).

tooth—what has been termed its talon—is in a very reduced state; in the Lemuroids, on the contrary, the *anterior* part is reduced, and the greater portion of the molar is composed by a part which, according to the trituberculate theory, is of a late development. Similar remarks apply to the superior molars. The obvious conclusion is that in two genera whose molars present a similar or almost similar form, this similarity may have been brought about without the cusps constituting the tooth being throughout homologous in the two forms; in other words, that we have to do with isomorphisms, not indicating true relationship.
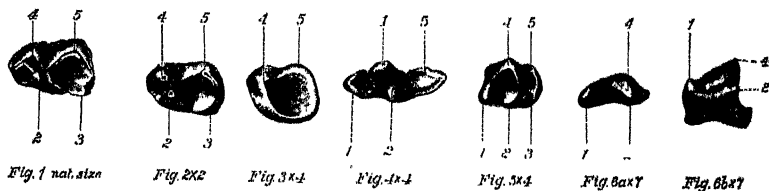
Fig. 1. *Megaladapis madagascariensis*, m_3, inf. dext.—Fig. 2. *Adapis magnus*, m_3, inf. dext.—Fig. 3. *Chirogale Milii*, m_2, inf. dext.—Fig. 4. *Eupleres Goudoti*, m_1, inf. dext.—Fig. 5. *Centetes ecaudatus*, m_1, inf. dext.—Fig. 6a. *Hemicentetes madagascariensis*, m_2, inf. dext. (from above as all the foregoing).—Fig. 6b. Same tooth, from the inner side. The outer cusps are numbered 4, 5; the inner cusps, 1, 2, 3.

With regard to the skull of *Megaladapis*, it may be remarked that the low cranium, with an almost straight upper profile and an elongate facial portion, would be regarded by most palæontologists as primitive.[*] There are, however, several considerations which seem to indicate that in many cases it is in reality a highly specialized feature. Firstly, the increase of the facial portion of a skull, both in a vertical and a horizontal direction, is often obviously due in great part to the specialization of the teeth. The molars, generally speaking, are increased vertically and sometimes horizontally, and the canines are transformed into tusks and weapons of different kinds. Secondly, other facts of ontogeny and phylogeny also suggest the same conclusion; for any skull during growth from the immature state to old age[†] and any type of Mammalian skull (*e.g.*, the Ungulate) traced from Eocene times to the present day, almost invariably shows the gradual diminution of the cranial and the concomitant elongation of the

[*] KARL A. ZITTEL, 'Handbuch der Palæontologie,' I. Abtheilung, vol. 4, 1892, p. 22.—MAX SCHLOSSER, "Die Affen, Lemuren, Chiropteren, Insectivoren ... des europäischen Tertiärs, &c.," 'Beiträge zur Palæontologie Oesterreich-Ungarns und des Orients,' I., Wien, 1887, p. 8) : "Die Verkürzung der Kiefer und Vergrösserung der Schädelkapsel tritt bei allen Säugethierstämmen auf und ist überhaupt der Endzweck aller den Schädel betreffenden Veränderungen."

[†] HERMANN VON NATHUSIUS, 'Vorstudien für Geschichte und Zucht der Hausthiere zunächst am Schweineschädel,' Berlin, 1864, p. 8.—WILHELM LECHE, "Beiträge zur Anatomie des *Myrmecobius fasciatus*," 'Verhandlungen des Biologischen Vereins in Stockholm,' vol. 3, May, 1891, No. 8, p. 139.

facial portion. Finally, it is evident that the change just referred to continues with increasing age, from the fact that the first dentition is more generalized than the second one, and requires, as a consequence, shorter jaws; and as it is now proved that the first dentition is ontogenetically and phylogenetically the older,[*] it follows that the shortness of the facial region is the primitive condition. There may, of course, be instances in which the reduction of the facial part of the skull is partly a secondary specialization (*e.g.*, certain Primates), but this circumstance does not invalidate the general result.

Let us proceed to consider the remaining parts of the skull of the *Megaladapis*, with a view of ascertaining whether they be primitive or specialized. The small brain-cavity in vertical, longitudinal, and transverse directions, gives this animal, when compared with the existing *Lemuroidea*, the appearance of being more lowly organized, and we must look among Insectivora and Marsupialia for a similar condition. It may sound like a paradox when I advance that the small size of the brain cavity in the two groups just mentioned, may be partially an acquired character. I have however, strong authority on my side for the assumption that the existing Marsupialia " are greatly modified members of the metatherial type," and " that most, if not all, of the Australian forms are of comparatively late origin."[†] On examining a bisected skull of a Marsupial, we may see that the great reduction in size of the cerebral fossa is very largely due to the development, in the adult animal, of sinuses in the walls of the covering bones, so that the brain cavity appears not only relatively, but absolutely larger in somewhat younger animals. The same takes place in several families of Insectivora, *e.g.*, the *Centetidæ*, which are considered as some of the most lowly organized of placental Mammals, but which, to judge at least from their dentition, as well as from the suppression of the jugals, and other characters, are highly specialized; the specialization of the teeth being carried further in one of the genera, *Hemicentetes*, where they have undergone a retrogressive evolution.

In *Megaladapis*, the cerebral fossa is likewise much reduced in size, and moreover, the olfactory fossa is greatly constricted by the lateral development of aërial sinuses to such an extent that they protrude somewhat in the cerebral fossa, the result being a reduction in size of this part also of the brain cavity, and as an obvious consequence, a partial atrophy of cerebral substance must have taken place; both cause and effect were certainly wanting in the young animal. The inspection of the corresponding outer parts of the skull showed us a considerable elongation of the lateral parts of the frontalia, backwards from the orbits. This might have led to the conclusion, that compared with existing Lemuroids, in which this elongation is

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[*] W. LECHE, " Studien über die Entwicklung des Zahnsystems bei den Säugethieren," 'Morpholog. Jahrbuch,' vol. 19, 1892, pp. 530, 531.

[†] T. H. HUXLEY, " On the Application of the Laws of Evolution to the arrangement of the Vertebrata, and more particularly of the Mammalia," ' Zool. Soc. Proc.,' London, 1880, p. 656.

never met with, the cranial cavity of *Megaladapis* is very elongate in an horizontal direction ; but, as we have seen, the very reverse is the case. As shown by the semi-diagrammatic fig. 10 (Plate 7), the front part of the cerebral fossa is narrowed to such an extent by the two frontal sinuses, that the boundary between the cerebral and the olfactory fossæ is .reduced superiorly to a mere vertical fissure, and at the base to a small triangular opening. I know of no other instance amongst Mammalia of a similar constriction between the two fossæ.

V. SYSTEMATIC POSITION.

From the foregoing comparison of the characters of *Megaladapis* with the existing *Lemuridæ*, and their discussion, the conclusion arises as to the systematic position we must assign to it. Unique as the skull is in several respects, so that we are entitled to place the animal in a distinct family, the *Megaladapidæ*, a closer examination has shown us that it only carries farther several characters possessed by various groups of existing Lemuroidea, and by *Adapis* amongst the extinct.

In the conformation of the dentition, *Megaladapis* closely approaches two Malagasy genera of Lemurids (*Lepidolemur*, and more closely still some of the forms which are included in the genus *Chirogale*), so that there is some ground for the assumption that an equally close analogy with *Lemuridæ* will be shown by the anterior part of the dentition, which is not known for the present. Even if this should not prove to be the case, I do not expect that it would alter the conclusions as to its systematic position.

The type of the inferior molars of *Megaladapis* approaches equally, as has already been stated, those of *Adapis*.

In the conformation of the interorbital frontal region, *Megaladapis* bears the most relation to *Adapis* amongst extinct, and the *Indrisinæ*, especially *Propithecus*, amongst recent Lemuroidea.

There are two characters in the cranium of *Megaladapis* for which we failed to find analogies amongst existing Lemuroidea, viz., the exceedingly small and low brain cavity, and the prolongation of the lateral parts of the frontals backwards from the orbits. As to the first,.*Adapis* approaches somewhat *Megaladapis*, so far, at least, as having a *low* brain case ; but the cranial cavity of the former is considerably longer in proportion. I trust I have shown with sufficient evidence, that *Megaladapis* presents, in this respect, an instance of retrogression ; whereas the same character may be a primitive .one in *Adapis*.

As regards the backward prolongation of the lateral region of the frontals, not in the least a Lemuroid feature, we meet with this character in groups remote from the Lemuroidea, and from each other, viz., in the *Carnivora*, *Insectivora*, and *Marsu-pialia* ; and we have seen that it is the result of the developing of aërial sinuses ; so

that it reveals itself as a specialized character of secondary importance, which is totally, or almost totally absent in young animals.

It is to be anticipated that skulls of young specimens of *Megaladapis* will bear a much closer resemblance than the adult to the existing Malagassy *Lemuridæ*. These resemblances will prove to consist, *e.g.*, in a more rounded cerebral cranium, in the brain cavity being relatively much more, and probably even absolutely more voluminous than in the old specimen, as well as in a more shortened facial cranium. The post-orbital elongation of the frontals will be wanting.

By the name of *Megaladapis* no *close* approach to *Adapis* is implied; although I anticipate that younger specimens will show closer relation to *Adapis* as well. I consider *Adapis* to form another distinct family of Lemuroidea. As some palæontologists have assigned to this genus a more remote position from existing Lemuroids, a few words in support of my view will not be out of place here, as the argument bears close relation to the subject of this paper.

FILHOL proposed for this fossil genus a distinct group of the *Pachylémuriens*, on the ground that in its dentition it is nearly related to the Ungulata. To this may be objected, that by placing *Adapis* in a distinct family, sufficient account is taken of the more generalized character of its dentition, shown by the incisors and canines, and the greater number of premolars, as compared with existing Lemuroidea. On the other hand the researches of GRANDIDIER and MILNE EDWARDS have shown, that in their anatomical characters the existing Lemuroids bear curious relations to several Ungulates, so that the existing members would equally deserve the denomination of *Pachylémuriens*, which for this reason becomes superfluous. And the more so, as several existing Lemuroids approach, in the structure of their molars, some smaller members of the Eocene Lophiodont Ungulates, as well as *Adapis*.

A similar view of the question has long ago been held by Sir WILLIAM FLOWER, when much less of living and extinct Lemuroids was known than at present.[*]

SCHLOSSER has thought it advisable to establish for the genus *Adapis* and several American Tertiary Lemuroids a distinct sub-order, *Pseudolemuridæ*, whilst *Necrolemur* of the European Eocene, and *Anaptomorphus*, *Cynodontomys*, *Mixodectes* of the American, are united with the existing *Lemuridæ*.[†]

If *Adapis* deserves to be separated from these last on account of its dentition; *Necrolemur* has the same claims, and so has *Anaptomorphus*. There is no greater difference in their dentition between *Adapis* on the one hand, *Necrolemur* and *Anaptomorphus*, on the other, than there is between the existing sub-families *Lemurinæ* and *Indrisinæ*. So that, as long as all these fossil forms are not more completely known, it seems advisable to leave them in the one sub-order, Lemuroidea,

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[*] WILLIAM HENRY FLOWER, "Extinct Lemurina," 'Ann. Mag. Nat. Hist.,' 4th series, vol. 17, London, 1876, pp. 323–328.

[†] MAX SCHLOSSER, 'Die Affen, Lemuren,' &c., 1, p. 19, *et seq.*

which would include the following families, leaving aside the less perfectly known fossil forms :—

Lemuroidea ⟨
1. *Adapidæ* (extinct) : *Adapis.*
2. *Anaptomorphidæ* (extinct) : *Anaptomorphus, Necrolemur.*
3. *Lemuridæ* (recent).
4. *Megaladapidæ* (extinct) : *Megaladapis madagascariensis.*
5. *Chiromyidæ* (recent),
6. *Tarsiidæ* (recent).

VI. ASSOCIATED FAUNA.

Before proceeding to the question of the geological age, a brief review must be given of the Vertebrate remains found associated in the marsh of Amboulisatra. Those discovered by GRANDIDIER* are the following :—

Aepyornis.

Bones of different size, which ALPH. MILNE EDWARDS and GRANDIDIER† are disposed to ascribe to three species : *Aepyornis maximus, Aep. medius,* and *Aep. modestus.* The largest bones were associated under the first name with the enormous eggs found in Southern Madagascar, and the association has been generally taken for granted, the only grounds for this view being that up to the present date no bones of a larger bird had been forthcoming. Taking in consideration, on the one hand, the number and great variety of forms of the New Zealand *Dinornis,* and on the other, the circumstance that a systematic palæontological exploration of the island is still a *desideratum,* some caution as regards this question was not out of place, and DAWSON ROWLEY has long ago‡ expressed strong doubts with respect to the above association.

In a recent meeting (June 6, 1893) of the Zoological Society of London, the Hon. WALTER ROTHSCHILD exhibited two femora, a tibio-tarsus and a tarso-metatarsus of *Aepyornis,* quite recently sent over by Mr. LAST from the south-west coast of Madagascar. These bones exceed in size the largest hitherto known, and the exhibitor suggested that they more properly might be associated specifically with the large eggs.

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* MILNE EDWARDS, " Sur des découvertes zoologiques faites récemment à Madagascar par M. ALFRED GRANDIDIER," ' Comptes Rendus Ac. Sc.,' vol. 67, pp. 1165–1167, Séance du 14 déc., 1868.

† ALPH. MILNE EDWARDS et ALF. GRANDIDIER, "Nouvelles Observations sur les Caractères zoologiques et les affinités naturelles de l'Aepyornis de Madagascar"; ALPH. MILNE EDWARDS, ' Recherches sur la Faune Ornithologique éteinte des Iles Mascareignes et de Madagascar.' Paris, 1866–1873, p. 110.

‡ GEORGE DAWSON ROWLEY, "On the Egg of *Aepyornis;* the Colossal Bird of Madagascar," ' Zool. Soc. Proc.,' London, 1867, pp. 892–895.

On the other hand, from a few preliminary remarks by DAMES* on *Aepyornis* remains in the Berlin Museum, collected by HILDEBRANDT, at Sirabé, North-Betsileo (Central Madagascar), it appeared as probable that they represented an apparently new form of *Aepyornis*, of comparatively small dimensions. These remains, amongst which a pelvis deserves particular mention, have been recently described and figured by R. BURCKHARDT as *Aepyornis Hildebrandti.*†

Crocodilus.

A Crocodile, *Crocodilus robustus*, VAILL. et GRAND., originally believed to be extinct,‡ but later on discovered by HUMBLOT§ as actually existing in the great lakes of the interior. This Crocodile, stated to reach a length of 10 metres, is nearly related to the Indian *Crocodilus palustris*, LESSON.‖ A humerus of *Crocodilus* from Sirabé, in the Christiania Museum is supposed by DAMES¶ to belong to the same species.

Testudo.

Two gigantic Chelonians, *Testudo abrupta*, GRAND., and *Testudo Grandidieri*, VAILL.** The last mentioned form was discovered besides by GRANDIDIER, in " Couches Sablonneuses," at Etséré (South-West Coast),†† and the remains sent to the British Museum by Mr. LAST, and described by BOULENGER,‡‡ seem to come from the same locality, in a cave.

Hippopotamus.

Finally, the remains of about fifty specimens of *Hippopotamus* were discovered by GRANDIDIER at Amboulisatra, and described as *H. Lemerlei.*§§ Remains of *Hippo-*

* W. DAMES, " Vorlage eines subfossilen Crocodil-Humerus von Madagascar," ' Sitzungsber. der Ges. naturforsch. Freunde.' Jahrg. 1886, No. 5, Berlin, 1886, p. 68.

† R. BURCKHARDT, " Über Aepyornis," ' Palaeontologische Abhandlungen,' Neue Folge, vol. 2, Jena, 1893.

‡ A. GRANDIDIER et L. VAILLANT. " Sur le Crocodile fossile d'Amboulintsatro (Madagascar)," ' Comptes Rend. Ac. Sc.,' vol. 75, 1872, pp. 150-151.

§ L. VAILLANT, " Remarques sur le *Crocodilus robustus*, VAILL. et GRAND., de Madagascar," ' Compt. Rend. Ac. Sc.,' vol. 97, 1883, pp. 1081-1083.

‖ L. VAILLANT, *loc. cit.* G. A. BOULENGER, ' Catalogue of the Chelonians, Rhynchocephalians, and Crocodiles in the British Museum (Natural History).' New ed., London, 1889, p. 286.

¶ W. DAMES, *l.s.c.*

** MILNE EDWARDS, ' Sur des découvertes zoologiques,' *l.s.c.* ALFRED GRANDIDIER, " Madagascar," ' Bull. Soc. Géogr.,' 2, 1871, p. 91. L. VAILLANT, " Remarques complémentaires sur les Tortues gigantesques de Madagascar," ' Compt. Rend. Ac. Sc.,' vol. 100, 1885, pp. 874–877.

†† MILNE EDWARDS, ' Sur des découvertes zoologiques,' etc., *loc. cit.*

‡‡ G. A. BOULENGER, " On Remains of an extinct gigantic Tortoise from Madagascar (*Testudo Grandidieri*, VAILLANT)," ' Trans. Zool. Soc.,' vol. 13, pp. 305–311.

§§ MILNE EDWARDS *loc. cit.*

potamus have been likewise discovered in Central Madagascar. The German explorer, HILDEBRANDT, obtained skeletons of this Mammal from the salt-marsh of Sirabó (*i.e.*, " much salt"),* and forwarded them to the Berlin Museum.† KOKEN‡ and DAMES§ identify this *Hippopotamus* with a skeleton from the same locality, obtained by Norwegian Missionaries and sent to the Christiania Museum, where they have been described by GULDBERG under the name of *H. madagascariensis*.‖

The brief description of the *H. Lemerlei*, given by GRANDIDIER¶ does not enable us to decide for the present if the Sirabó specimen is identical with the *H. madagascariensis*, GULD. ALPH. MILNE EDWARDS is of opinion** that the *H. Lemerlei* from Amboulisatra comes very near to the *II. liberiensis (Choeropsis)*, a living form from Liberia. The *Aepyornis* remains from the central parts being different from those of the south-west coast, and it being doubtful at present if the age of the two deposits, which we shall have to discuss hereafter, is the same, there is an *à priori* assumption against the identification of the respective *Hippopotamus* remains. The question becomes more puzzling still by the circumstance that a cranium of *Hippopotamus* in the British Museum, found in a swampy deposit at Sirabé††—the same district from which were obtained the remains in the Berlin and Christiania Museums—is decidedly different in some essential points from the one described by GULDBERG. For some further particulars on this question, I refer to the following chapter.

* J. M. HILDEBRANDT, "Skizze zu einem Bilde central-madagassischen Naturlebens im Frühling," ' Zeitschr. d. Ges. f. Erdkunde zu Berlin,' 16; Berlin, 1881, pp. 194–203. R. BARON, " Notes on the Geology of Madagascar." 'Quart. Journ. Geol. Soc.,' London, vol. 45, 1889, p. 308, where the name is written " *Antsirabe.*"

† KOKEN, "Reste eines subfossilen Hippopotamus, *Hippopotamus madagascariensis*, GULDBERG," ' Sitzungsber. Ges. Naturf. Freunde.' ' Berlin, 1886, p. 55. W. DAMES, *l.s.c.*

‡ *Loc. cit.*

§ *Loc. cit.*

‖ G. A. GULDBERG, "Undersögelser over en subfossil flodhest fra Madagascar," ' Forhandlinger i Videnskabs Selskabet i Christiania, aar 1883.' Christiania, 1884, No. 6.

¶ MILNE EDWARDS, ' Sur des découvertes zoologiques,' *loc. cit.*

** *Loc. cit.*

†† R. BULLEN NEWTON, " On the Discovery of a Secondary Reptile in Madagascar, *Stenosaurus Baroni* (*n. sp.*) ; with a reference to some Post-Tertiary Vertebrate Remains from the same country, recently acquired by the British Museum (Natural History)." ('Geolog. Magazine,' Decade III., vol. 10, No. 347, May, 1893, p. 197.) Mr. B. NEWTON draws besides attention to the fact that a few *Hippopotamus* teeth from Madagascar were sent to this country as long as sixty years ago, and are preserved in the Museum of the Geological Society, the label accompanying stating them to come from a locality thirty miles from Antananarivo, which is the capital, situated in Central Madagascar. The brief reference in the 'Proc. of the Geol. Soc. of London,' (vol. 1, No. 31, 1833, p. 479), runs as follows :— " A letter was afterwards read from Mr. TELFAIR to Sir ALEXANDER JOHNSTON, V.P.R.A.S., accompanying a collection of recent conglomerate rock, from the Island of Madagascar, containing fragments of a tusk and part of a molar tooth of a Hippopotamus, and communicated by RODERICK IMPEY MURCHISON, Esq., F.G.S."

The small collection obtained from the marsh of Amboulisatra by Mr. LAST, consists, besides the skull of *Megaladapis*, of two occipital portions and bones of *Hippopotamus*.

Part of the pelvis of *Sus* which, from its fresh appearance, seems to be more recent than the other remains.

Vertebræ of a species of *Potamochærus*, which may prove to be the one actually existing in Madagascar.

Some remains of *Aepyornis*, and of other smaller birds.

Bones and fragments of the carapace of *Testudo Grandidieri*, VAILL., identified by Mr. BOULENGER.

The collection contains besides fragmentary bones of some Mammals, but so few in number, and at the same time so enigmatical, that the safer course to pursue will be to say as little as possible about them for the present. A femur of a young individual—the distal epiphyses are wanting—with a very strong lesser and a feebly developed third trochanter, presents, by its antero-posterior flattening, a striking resemblance with a *Manis* femur, with which however, for other reasons, I do not feel inclined to unite it; but it may be a Rodent or an Insectivore. The length of the bone, so far as preserved, is 135 millims.

VII. GEOLOGICAL AGE.

We have next to meet the question as to the geological age of the cranium which forms the subject of the present inquiry.

From Mr. LAST's correspondence it appears that the bones from the marsh of Amboulisatra were found lying between a stratum of a "white clayey substance" above, and a deposit of "greenish sand and stones" below. The white clay has a thickness of 18 inches to 2 feet, and is overlaid by 6 inches of black soil. The matrix adhering to the bones is in fact a green quartzitic sand. They have a very fresh appearance, and in their mode of preservation and colouring much resemble the animal remains from lake-dwellings, some of them having an even fresher appearance.

With regard to the remains of *Crocodilus robustus* from the marsh of Amboulisatra, it has already been stated that this species is actually existing in the lakes of the interior.

ALPH. MILNE EDWARDS and GRANDIDIER, in their paper on the remains of *Aepyornis* from this same deposit, sum up in the following words the discussion as to their age: "Il ressort donc clairement de ces faits que l'*Aepyornis* a vécu à une époque où l'homme habitait déjà Madagascar, mais que, dépourvu de moyens de défense et probablement aussi d'intelligence, il a été rapidement détruit, et que les voyageurs des xvi° et xvii° siècles n'ont pu que recueillir sur son compte les souvenirs

déjà anciens et, par conséquent, mêlés de merveilleux que les tribus sauvages se transmettaient de génération en génération."*

Reference is here made, first, to the legendary bird Roc or Ruc, mentioned by MARCO POLO and other travellers, influenced by which Professor BIANCONI, of Bologna, has endeavoured to prove in several Memoirs that the *Aepyornis* was a bird of prey allied to the Vulture.[†]

The legends referring to gigantic birds may not necessarily be considered as a proof of their contemporaneity with Man, as they might have been originated by the view of the enormous eggs. In the same manner as the fables relating to monsters and amazons inhabiting the island of Samos, and which are reported by ÆLIANUS and PLUTARCH, owe their origin to the acquaintance of the ancient Greeks with the great quantity and occasionally enormous size of (Miocene) bones met with in that island.[‡]

The opinion that the *Aepyornis* was contemporaneous with Man is, however, strengthened by the fact stated by MILNE EDWARDS and GRANDIDIER, viz., that on one of the tibiæ, " On voit à l'extrémité supérieure des empreintes profondes pratiquées à l'aide d'un instrument tranchant ; il semble évident que ces incisions ont été faites en coupant les ligaments du genou pour séparer les os de la jambe de celui de la cuisse, et elles décèlent la main de l'homme."[§]

Moreover, on one of the fragments of metatarsus are to be seen, according to the same authors, some superficial cuts or scratches (*incisions*), very similar to those found sometimes on bones from caves and considered to be the work of Man.[||]

On the mandibula of the *Megaladapis* scratches are also seen which have the appearance of age, and which seem to have been produced by some cutting instrument ; but they may also be referred to the action of sharp-pointed teeth.

Most of the eggs of the *Aepyornis* have been reported to be found on the seashore of South Madagascar, " on the abrupt rise of the dunes, even on the surface of the sand, when there is a crumbling of the earth, or when tropical rains heave up parts of the sand."[¶] The dunes are considered both by GRANDIDIER[**] and the Italian

* ALPH. MILNE EDWARDS et ALF. GRANDIDIER, "Nouvelles Observations sur les caractères zoologiques et les affinités naturelles de l'*Aepyornis* de Madagascar," *loc. cit.*, p. 112.

† BIANCONI, "Dell' *Epyornis maximus* menzionate da Marco-Polo e da Fra Mauro," 'Memorie dell' Accademia dello Scienze,' Bologna, 1862; *ibid.*, "Degli scritti di Marco-Polo e dell' uccello Ruc da lui menzionato," *loc. cit.*, 2ª serie, vol. 2, Bologna, 1862; *ibid.*, " Studi sul Tarso-Metatarso degli uccelli ed in particolare dell' *Epyornis maximus*," *loc. cit.*, 23 Aprile, 1863, e 12 genn., 1865.

‡ C. I. FORSYTH MAJOR, " Le Gisement ossifère de Mitylini," 'Extrait de "Samos," étude géologique, paléontologique et botanique,' par C. DE STEFANI, C. I. FORSYTH MAJOR, et W. BARBEY, Lausanne, 1892, pp. 1–2.

§ *Loc. cit.,* p. 112.

|| *Ibid.*

¶ ALF. GRANDIDIER, "Observations sur le gisement des œufs de l'*Epyornis*," 'Comptes Rendus Acad. Sc.,' Paris, vol. 65, 1867, pp. 476–478. GEORGE DAWSON ROWLEY, "On the Egg of *Aepyornis*, the Colossal Bird of Madagascar," 'Zool. Soc. Proc.,' London, 1867, p. 892.

** GRANDIDIER, *loc. cit.* ROWLEY, *loc. cit.*

geologist, CORTESE,* to be modern alluvia, the deposition of which is still in progress. So that here we have another proof that the Bird to which they pertain was still in existence at a recent period, later probably than the Pleistocene.

It would seem, however, that the eggs were more frequently obtained from some marshes. This circumstance seems to have been kept secret; the eggs, though rare, having become, on account of the high price paid for them by collectors, a sort of article of exportation from Madagascar. CORTESE† was the first to refer to their being found in marshes, and the geologist, PÉLAGAUD, to whom the Bologna Museum owes an egg of *Aepyornis*, and who has more than once visited Madagascar, informed Professor CAPELLINI as to the places where they are found and the methods resorted to in seeking for them.‡

To account for the presence of the eggs in the marshes, we must suppose that the water has only recently invaded the sandy region where they were originally deposited by the Birds; this, according to CAPELLINI,§ would be in relation with the gradual subsidence of the west coast of Madagascar, as admitted by CORTESE.‖

Further arguments in favour of a recent origin of the bones occur in connection with the discovery of those of *Hippopotamus* in Central Madagascar.

HILDEBRANDT, who was the first to discover remains of this Mammal¶ in the salt marsh of Sirabé, states that the natives have various names for it, one of them being *Laloména*, which means, according to HILDEBRANDT, "the smooth red one."**

Dr. BORCHGREVINK, who was an eye-witness of the discovery of the *Hippopotamus* skeletons, now in the Christiania Museum, has given a report of this discovery in the

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* E. CORTESE, "Appunti geologici sull' isola di Madagascar," ' Boll. del R. Comitato Geologico d' Italia,' vol. 19, Roma, 1888, pp. 118, 119.

† "La parte più meridionale dell' isola, cioè l'Antanosy, l' Antandroy e il Tanala, è bassa, paludosa in gran parte inesplorata. Pare che negli stagni di quella regione si trovino i gusci delle gigantesche uova di *Aepyornis*." E. CORTESE, "Appunti geologici sull' isola di Madagascar," 'Boll. del R. Comitato Geologico d' Italia,' vol. 19, Roma, 1888, p. 119.

‡ GIOV. CAPELLINI, "Sul primo uovo di *Aepyornis maximus* arrivato in Italia," 'Memorie R. Accad. delle Scienze dell' Ist. di Bologna,' serie iv., vol. 10, Bologna, 1890. In a letter transcribed on p. 26, M. PÉLAGAUD states :—" Vous savez que tous ces œufs ont été trouvés dans un marais." With reference to the egg sent to Bologna he writes (p. 27) :—" Il vient des environs de Nos-Vey côte sud-ouest de la grande terre. Vous savez comme on les découvre. Des chercheurs sondent à coup de lance (sagaie) la vase des deltas de certaines rivières marécageuses jusqu'à ce qu'on rencontre un corps dur . . ."

Thirty years ago Professor TENNANT exhibited to the Zoological Society of London a very perfect egg of the *Aepyornis* (lent to him for exhibition by M. J. F. BRUNET), which was stated to have been obtained in Madagascar, "at a depth of 50 feet, in digging a mine of iron," (' Zool. Soc. Proc.,' London, 1863, p. 438.)

§ *Loc. cit.*, p. 35.

‖ *Loc. cit.*, p. 127.

¶ Now in the Berlin Museum.

** J. M. HILDEBRANDT, *loc. cit.*, p. 197.

'Transactions of the Christiania Scientific Society.'[*] He, too, mentions, in connection with the *Hippopotamus*, a legendary monster, *Lalimena*, provided with large sharp tusks. According to Dr. BORCHGREVINK, the meaning of this word is not certain; perhaps it signifies "the red one who dives deeply" (*den Roede, som gaar dybt*), or "the red fly" (*den roede Flue*) (?). Some accounts state this animal to have resembled a huge boar; and tradition says that it was one of the prerogatives of the sons of kings to fight, or, as the tales have it, to *play* with, this monster.

The skeleton in question has been fully described by G. A. GULDBERG.[†]

From SIBREE, one of the editors of the 'Antananarivo Annual,' we learn that the remains of *Hippopotamus* obtained by the Museums of Berlin and Christiania were discovered during the excavations for the foundations of a bath-house, erected by the Norwegian Mission over the hot spring of Antsirabé; adding that there have been occasional vague reports of the existence of some huge animal in the southern parts of the islands, and suggesting that possibly the *Hippopotamus* is not yet extinct there, and that the half-mythical stories of the *Sungòmby*, *Tòkandìa*, *La?mèna*, and other strange creatures, current among the Malagassy, are traditions of the period when these huge Pachyderms were still to be seen in the lakes and streams and marshes of Madagascar.[‡]

In addition to these informations, it may be worth mentioning that FLACOURT, "Directeur Général de la Compagnie Françoise de l'Orient," who resided for years in the Fort Dauphin, erected by the French on the south-east coast of Madagascar, as a "Commandant pour sa Majesté dans la dite Isle et és Isles adjacentes," published in 1658 a 'History of Madagascar.' In this work two chapters are dedicated to the fauna of the island, giving the native names of the animals with brief descriptions,[§] part of which are easily recognizable, their names having been confirmed by recent travellers. Besides these, FLACOURT names and describes four quadrupeds which, from the size as well as other characters assigned to them, cannot be identified with any known existing Malagassy Mammals.[||] In some instances the author wrote only

[*] CHR. BORCHGREVINK, "Oplysninger om et paa Madagascar fundet subfossilt Flodhest-Skelet." ('Oversigt over Videnskabs-Selskabets Moeder i 1882,' den 12 Mai; 'Mathematisk-Naturvidenskabelig Classe,' pp. 8–11; 'Forhandlinger Videnskabs-Selskabet i Christiania Aar 1882,' Christiania, 1883.)

[†] *L. s. c.*, p. 24.

[‡] JAMES SIBREE, jun., "The Volcanic Lake of Tritriva: its Physical Features and Legendary History." ('The Antananarivo Annual and Madagascar Magazine,' Antananarivo, 1888, No. 12 (part 4 of vol. 3), pp. 468, 469.

[§] "Histoire de la grande Isle Madagascar, composée par le Sieur DE FLACOURT, Directeur Général de la Compagnie Françoise de l'Orient, et Commandant pour sa Majesté dans ladite Isle et és Isles adjacentes. Avec une Relation de ce qui s'est passé és années 1655, 1656, et 1657, non encor veuë par la première Impression." Chap. 38, "Des Animaux terrestres et des Insectes," pp. 151–159. Paris, 1661 (the first edition is of 1658).

[||] *Loc. cit.*, pp. 154, 155. "*Tretretretre* ou *Tratratratra*, c'est un animal grand comme un veau de deux ans, qui a la teste ronde, et une face d'homme; les pieds de devant comme un singe, et les pieds de

from hearsay, and may have reported some of the more or less fabulous accounts of the natives. However, FLACOURT'S information deserves mention, especially as the general trustworthiness of his statements has been acknowledged by modern explorers.*

The last set of Vertebrate remains, discovered by Mr. LAST on the south-west coast of Madagascar, without indication of the exact locality, and received in England in the beginning of June of this year, contains, besides the bones of *Aepyornis* of exceptional size, the following remains :—

1. An incomplete skull and mandibula, besides numerous bones and isolated teeth of *Hippopotamus*.

2. A skull, two humeri, as well as other bones of *Crocodilus robustus*.

3. Several mandibular rami and numerous bones of a slender-legged form of *Bos*.

The association of the last-mentioned genus goes far to prove that the extinct Vertebrata recorded, co-existed in the island with domestic cattle; the bones of the *Bos* presenting the same coloration as part of the other remains; whilst those of the *Aepyornis*, and several of those of the *Hippopotamus* show even a fresher appearance.†

derrière aussi. Il a poil frisoté, la queue courte et les oreilles comme celles d'un homme. Il ressemble au *Tanacht* descrit par AMBROISE PARÉ. Il s'en est veu un proche l'estang de Lipomani aux environs duquel est son repaire. C'est un animal fort solitaire, les gens du païs en ont grand peur et s'enfuient de luy comme luy aussi d'eux.

" *Antamba*, c'est une bête grande comme un grand chien qui a la teste ronde, et au rapport des Nègres, elle a la ressemblance d'un Leopart, elle devore les hommes et les veaux. Elle est rare et ne demeure que dans les lieux des montagnes les moins frequentez.

" *Mangarsahoc*, c'est une bête fort grande qui a le pied rond comme un cheval et les oreilles fort longues. Quand elle descend d'une montagne elle a de la peine à voir deuant elle, à cause que ses oreilles luy cachent les yeux, elle fait un grand cry à la façon d'un asne. Je crois que ce pont estre un asne sauvage. Il y a une montagne à vingt lieuës du fort Dauphin que les François ont nommé *Mangarsahoc* du nom de cette animal, y on ayans autres fois oüy crier un.

" *Breh*, c'est un animal que les Nègres de Manghabei disent estre dans le païs des Antsianactes, qui a une corne seule sur le front, grande comme un grand cabrit, et est fort sauvage, il faut que ce soit une licorne."

When announcing the discovery of the *Aepyornis* eggs, ISID. GEOFFROY-SAINT-HILAIRE quotes the following passage from FLACOURT, referring to a bird called *Vouron patra :*—" C'est un grand oyseau qui hante les *Ampatres*, et fait des œufs comme l'Autruche; c'est une espèce d'Autruche. Ceux des dits lieux ne le peuvent prendre, il cherche les lieux les plus déserts " (*loc. cit.*, p. 165). FLACOURT'S Vouron patra has been supposed by GEOFFROY-SAINT-HILAIRE and other writers to be referable to one of the minor forms assigned to the genus *Aepyornis*, and in the first line to the fragmentary egg-shells, as mentioned by P. GERVAIS : "qui paraissent avoir dû être du volume de ceux des Autruches." (ISID. GEOFFROY-SAINT-HILAIRE, *l. s. c.*; P. GERVAIS, *l. s. c.*)

* See ALF. GRANDIDIER, Madagascar (*loc. cit.*, p. 82) : " *L'Histoire de Madagascar*, par FLACOURT, seule, porte le cachet de la vérité; ce que le gouverneur de Fort-Dauphin écrivait, en 1661, sur la petite peuplade des Antanosses, est vrai encore de nos jours."

† The following account of the Madagascar cattle in the 17th century, as given by FLACOURT,

The evidence contained in the foregoing pages, shows, with a sufficient degree of certainty, that the Vertebrate remains discovered in Madagascar belonged to animals, part, if not all, of which have been seen by Man at a relatively recent date.

There is however, besides, another piece of information, though scanty for the present, which entitles us to look forward towards the discovery of a Mammalian fauna, geologically older than the one treated of in the foregoing.

From the few papers published on the geology of Madagascar, which at the present day, according to GRANDIDIER,* possesses but a scarce number of lakes, it appears that numerous old lake-bottoms exist in the central part, as well as terraces of lacustrine origin.† From the correspondence of Mr. LAST, who spent last autumn and the whole of the winter in the Antinosy country, we gather that in this almost unexplored region of Southern Central Madagascar, the beds of old lakes equally abound. Mr. LAST further states as follows : "Now the lakes are all dry, leaving a bed of grey, marly clay, from three to five feet thick, and near the bottom of this the isolated bones are found." Another formation, termed by the writer a "soft red sandstone," seems to overlay in many places the grey marl ; in one place where the river Itungansuba has cut a passage through these various deposits, a section of them is seen. So that the suspicion arises that the grey marl of the interior is not the equivalent of the white clay in the marsh of Amboulisatra near the sea-shore, but rather belongs to a geologically older formation. CORTESE mentions‡ that in the central region, extensive stratified deposits of red arenaceous clays (*argille sabbiose rosse*) exist resulting from the decomposition of the gneissic rocks. These red arenaceous clays he ascribes to the Pleistocene. If they are the same formation with Mr. LAST's "soft red sandstone" of the southern central parts, then the underlying grey marls and the fossils they contain, may be Tertiary.

Whatever may hereafter prove to be the case it appears *à priori* highly improbable that the formation of lake basins has been limited to the Post-Tertiary period. Whilst from the presence, in one or more districts, of lignite,§ believed by CORTESE

acquires a peculiar interest in relation with the above statement. " Il y a de trois sortes de bœufs en ce païs sçauoir ceux qui ont des cornes ; d'autres qui se nomment *Boury*, qui ont la teste ronde, et n'ont point de cornes, et d'autres qui ont des cornes pendantes attachées à la peau de la teste seulement " (?), (probably *Bubalus*) " et tous ont de grosses louppes de graisse sur le chinon du col, de laquelle louppe l'on en fond la graisse pour manger au lieu de beurre : d'autant qu'elle est aussi agreable que le beurre. Ces bœufs quelques gras qu'ils soient, ont très-peu de suif, ce qu'il y en a est très-bon à faire de la chandelle. Il y a encores dans le païs des Machicores, ruiné des guerres, une grande quantité de bœufs sauuages qui n'ont point de louppes. Ils sont comme ceux d'Europe : Toutesfois sont hauts de jambe, et courent par trouppe comme des cerfs." (DE FLACOURT, *loc. cit.*, p. 151.)

　* ALF. GRANDIDIER, "Madagascar" ('Bull. Soc. Géogr.,' vol. 2, 1871, p. 104.)

　† M. CORTESE, 'Appunti Geologici,' &c., pp. 106, 107, 108. R. BARON, *loc. cit.*, pp. 306-308.

　‡ 'Appunti geologici,' p. 117.

　§ E. CORTESE, "Osservazioni geologiche sul Madagascar," 'Boll. del R. Com. Geol.,' vol. 18, 1887, p. 187. *Ibid.*, 'Appunti Geologici,' &c., *loc. cit.*, p. 123. R. BARON, *loc. cit.*, p. 325.

to be of Pliocene age, it becomes further almost a certainty that lacustrine deposits of Tertiary age exist in Madagascar. I anticipate accordingly that a Tertiary Vertebrate fauna will sooner or later be forthcoming in the island.

With regard to this proposition I wish to call attention to another circumstance. In the foregoing inquiry reference was made to a paper by GULDBERG, containing a detailed description, with plates, of the *Hippopotamus* from Sirabé, in the Christiania Museum. GULDBERG supposes that this form may be identical with the *H. Lemerlei*, whose remains were discovered by GRANDIDIER in the Amboulisatra marsh. The provisional description given by the French author[*] does not allow us to pronounce with certainty on the matter, so that, being in doubt, GULDBERG prefers to assign a new specific name to the Sirabé remains (*H. madagascariensis*).

The *Hippopotamus* skull in the British Museum comes, as above stated, from this same district of Sirabé ; I was in consequence, as a matter of course, prepared to find it agreeing with the one described by GULDBERG. However, a close examination proved quite the contrary. It can only be said, in a general way, that both are somewhat intermediate between the lower pliocene *H. sivalensis* and the existing *H. amphibius*, a remark which likewise applies to *H. Lemerlei*; but otherwise the skull in the British Museum differs from the one described by GULDBERG in various respects, some of the variations being such that it is impossible to account for them as being due to age or sex. The London skull appears to be equally different from those described by GRANDIDIER in some points in which these last agree with the Christiania skull, so far at least as one may be allowed to judge from GRANDIDIER'S description. Now, as it seems at first sight difficult to admit that two different forms of *Hippopotamus* existed in the same district, I feel almost inclined to conclude that the differences alluded to may be due to the fact of the two skulls from Sirabé being of a different geological age.

However, I do not wish to insist too strongly on this point so long as the Paris remains have not been submitted to a close comparison, in which would have to be included as well, the remains sent to Berlin by HILDEBRANDT.

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[*] MILNE EDWARDS, "Sur des découvertes zoologiques faites récemment à Madagascar par M. ALFRED GRANDIDIER," 'Comptes Rendus Acad. Sc.,' vol. 67, séance du 14 Déc., 1868, pp. 1165–1167.

DESCRIPTION OF PLATES.

All figures natural size.

PLATE 5.

Megaladapis madagascariensis, gen. et. sp. nov.

Fig. 1. View of the right side of the skull.
Fig. 2. Outside view of the right mandibular ramus.
Fig. 3. Front view of the same, to show the symphysis.

PLATE 6.

Megaladapis madagascariensis.

Fig. 4. Upper view of the skull.
Fig. 5. Occipital view.   The constriction between the cerebral and the olfactory
fossa is visible through the foramen magnum.
Fig. 6. Fragment of the right maxilla of a second specimen, from below, showing the
two posterior molars and the alveoli of the first molar, as well as of the
two posterior premolars.
Fig. 7. Upper view of the inferior molars and posterior premolar (right mandibular
ramus, fig. 2).

PLATE 7.

Megaladapis madagascariensis.

Fig. 8. Inferior view of the skull.   *ms.,* backward prolongation of the inflated
maxilla, which on the left side is broken, so that the maxillary sinus is
visible.
Fig. 9. Internal view of the right mandibular ramus.
Fig. 10. Diagram of the boundary between the cerebral and the olfactory fossæ, as
seen through the foramen magnum.   The posterior ridge of the lamina
perpendicularis of the mesethmoid (crista galli) is visible through the
opening.

n:

Fig.3.

Fig.2.

Fig. 4.

Fig. 6.

m^3 m^2 (m^1) (p^4) $(p$

Fig. 7.

p^4 m^1 m^2 m
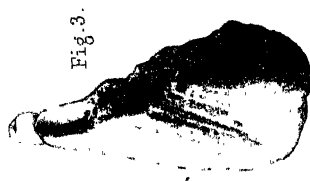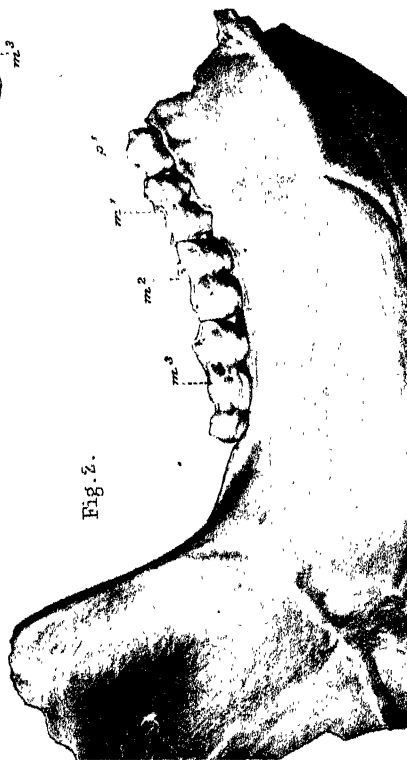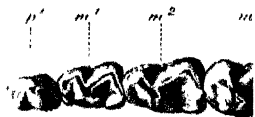
Fig. 5.

Fig.9.

Fig.10.

III. *A Further Minute Analysis by Electric Stimulation of the So-called Motor Region (Facial Area) of the Cortex Cerebri in the Monkey* (Macacus sinicus).*

By Charles E. Beevor, *M.D., F.R.C.P., and* Victor Horsley, *M.B., F.R.C.S., F.R.S. From the Laboratory of the Brown Institution and from the Pathological Department of University College, London.*

[Plates 8, 9.]

Contents.

INTRODUCTION.

In continuation of our former minute analysis of the excitable region of the cerebral cortex, we have explored the so-called centres for the facial, lingual, and pharyngeal movements, or as we prefer to speak of them collectively, the facial area. This district, as will presently be seen, has been mapped out by numerous investigators, and its general limits are fairly well understood; but as we have found in the course of our investigations several points untouched, especially relating to the

representation of the movements of the tongue, we think it better to arrange the facts previously determined in an historical introduction and to subjoin our own observations. In this, as in our second paper* on the minuter representation of movements in the cerebral cortex, we have, in order to avoid discrepancies in the arrangement of the sulci, employed only the same variety of Monkey, viz., *Macacus sinicus*. In all we have performed twenty experiments.

HISTORICAL INTRODUCTION.

FRITSCH† and HITZIG, in the original memoir which forms the basis of all modern research on the subject, contented themselves with defining the foci of representation of movements of the face in the Carnivora.

HITZIG‡ later investigated the point in the Monkey. In his paper on equivalent regions of the brain in Dogs, Monkeys, and Men, HITZIG describes an excitation experiment on a Monkey (*Innus rhesus*). The representation of facial movements he obtained in this case was as follows :—The highest point of the facial "region" was "centre 3" and is figured by him as just above the genu of the fissure of ROLANDO and in the ascending frontal gyrus. At this point he obtained closure of the eye and retraction of the ear. Below this point, excitation elicited, in addition to the ear movement, contraction of the masseters, then movement of the lips, and finally, at a point just above the fissure of SYLVIUS, the current produced opening (*aufsperren*) of the mouth. Just above this latter place he obtained retraction of the angle of the mouth, and in neighbouring parts movements of the tongue muscles, depressors of the jaw, elevators and depressors of the hyoid bone. All these latter-named movements were *bilateral*.

FERRIER.§—FERRIER, in the Monkey, determined four points in which are represented movements of the facial muscles and tongue, as follows :—

No. 7. (Situated on the ascending frontal gyrus apparently just opposite and below the genu of the fissure of ROLANDO, see further, "Anatomy," p. 4), "Retraction and Elevation of the Angle of the Mouth and the Action of the Zygomatic Muscles."

No. 8. (Situated just below and above sulcus *v*, see further, "Anatomy," p. 4), "Elevation of the Ala of the Nose and Upper Lip, Associated with Depression of the Lower Lip so as Fully to Expose the Canine Teeth."

No. 9. (Situated on the lower extremity of the ascending frontal gyrus immediately in front of the sulcus *v*, see further, "Anatomy," p. 4), "Opening of the Mouth with Protrusion of the Tongue."

No. 10. (Situated on the lower end of the ascending frontal gyrus between the

* 'Phil. Trans.,' B, 1888.
† 'Archiv für Anat. und Phys.' (DU BOIS-REYMOND), 1870, p. 300.
‡ 'Untersuchungen über das Gehirn,' Berlin, 1874, p. 132.
§ 'The Functions of the Brain,' 1st ed., 1876, 2nd ed., 1886.

lower end of the fissure of ROLANDO and v, see further, " Anatomy," p. 4), " Opening of the Mouth with Retraction of the Tongue." "These movements" (described under Nos. 9 and 10) "are occasionally repeated for some time after the electrodes are withdrawn. The movements are bilateral."

No. 11. (Situated on the lower extremity of the ascending parietal gyrus covering the space between the hinder end of the fissure of ROLANDO and the lower end of the intraparietal sulcus,) " Retraction of the Angle of the Mouth." "The action is that of the platysma myoides, and when this is strong the head is drawn slightly to the side."

H. MUNK* confirmed the fact that the movements of the face and tongue were represented in the Monkey in the area delineated by HITZIG and by FERRIER, but did not record minuter details of the localisation.

SCHÄFER and HORSLEY.†—These investigators found on sub-dividing the facial area, as marked out by FERRIER, that the broad facts described by him were correct, except that the *upper* part of the area was the seat of elevation of the angle of the mouth, and that the *lower* half was the region of retraction of the same. (It may be noted that FERRIER, in the last chapter of his book, when he transfers the excitation results from the Monkey's brain to that of Man, places these areas of representation in the reverse order, *i.e.*, the same as SCHÄFER and HORSLEY, and, as will presently be seen, the same as we have found it, namely, "Elevation" above "Retraction.) They further observed that just below the upper border of the so-called face region, *i.e.*, just opposite to the lower end of the intraparietal sulcus, the movements of winking and of closure (synchronous) of both pairs of eyelids were therein represented; also that half-way down the vertical limit of the præcentral sulcus and just below it, the movement of drawing in and out of both cheeks was elicited on excitation; also that pouting and pursing of the lips were evoked from the horizontal strip of cortex running forwards from the lower end of the fissure of ROLANDO towards the præcentral sulcus; finally, from above the Sylvian fissure and between the lower end of the fissure of ROLANDO and " v," it was found that the tongue was actively retracted and also directed towards the side of excitation.

ANATOMY.

The excitable region of the cortex which we have investigated in this research comprises about the lower third of the ascending frontal and parietal convolutions and the commencement of the supramarginal gyrus. More exactly, the area now under consideration is bounded below by the fissure of SYLVIUS, in front by the

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* 'Ueber die Funktionen der Grosshirnrinde. Gesammelte Mittheilungen,' 2te Auflage, 1890, p. 53, also ' Real-Encyclopädie d. Gesammten Heilkunde.'

† 'Phil. Trans.,' 1888 (paper read February, 1887). For certain minute details see also SCHÄFER, ' Ueber die Motorischen Rinden-Centren des Affen-Gehirns,' Festgabe z. CARL LUDWIG gewidmet, 1887.

vertical limb of the præcentral sulcus, and by a line drawn from the inferior end of this latter vertically down to the Sylvian fissure, behind by a line drawn from a point just behind the anterior end of the intraparietal sulcus vertically downwards to the Sylvian ·fissure; and superiorly by a line drawn horizontally across the ascending frontal convolution on a level with the upper extremity of the præcentral sulcus above the bend in the fissure of ROLANDO, called the genu (inferior genu), and by a line drawn across the ascending parietal convolution from the genu of the fissure of ROLANDO to the anterior end of the intraparietal sulcus.

In our paper ('Phil. Trans.,' B., 28, 1888) on the minuter representation of the movements of the limbs, we have described and figured the anatomy of the excitable region, with a minor exception, which we must now notice. It will at the same time, perhaps, be useful to enumerate the chief points before described. Reference, moreover, to fig. 1 (Plate 8) will render this more easy.

The mode of termination and form of the fissure of ROLANDO we have elsewhere* fully described, and need, therefore, only allude to the inferior genu, as showing the position of the borderland between the areas for representation of the upper limb and of the face.

The præcentral sulcus we have fully described in our first paper in the 'Philosophical Transactions,'* but we would here refer to two small sulci in the ascending frontal and parietal convolutions respectively. In the ascending frontal convolution, at its lower part, there is a small vertical sulcus, *st* in fig. 1, about 3 millims. long, the lower end of which is about 4 millims. from the fissure of SYLVIUS, and it is situated about 8 millims. behind the præcentral sulcus and 5 millims. in front of the fissure of ROLANDO. This sulcus, which is very constant in *Macacus sinicus*, was figured in a previous paper† of ours as "*v*," and was described by SEMON and HORSLEY‡ as forming the posterior border of the area for the more absolute representation of the vocal cords. In the ascending parietal convolutions there is also a slightly marked sulcus, *rct* in fig. 1, about 2 millims. long, situated vertically about 2 millims. behind the fissure of ROLANDO, and the same distance above the fissure of SYLVIUS and below the anterior end of the intraparietal sulcus. This small sulcus, which is not so constant as the sulcus "*v*," we propose to call "*u*."

These two small-subordinate sulci are termed by EBERSTALLER, in Man, the sulcus transversus frontalis inferior and the sulcus retrocentralis transversus respectively.

We are inclined to adopt this nomenclature for the lower Monkey also. The point is specially discussed by Professor CUNNINGHAM in his memoir "A Contribution to the Surface Anatomy of the Cerebral Hemispheres" (Royal Irish Academy, Dublin, 1892).

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* 'Phil. Trans.,' vol. 178 (1887), B, 6, p. 154.
† 'Phil. Trans.,' vol. 179 (1888), B, p. 206.
‡ 'Phil. Trans.,' vol. 181 (1890), B, p. 197.

METHOD OF INVESTIGATION, INCLUDING NOTATION.

Operation and Method of Recording.—In all cases we have exposed the cortex cerebri, with the precautions against cooling, drying, &c., described in our previous papers. After the opening of the dura mater the sulci were, as before, carefully plotted out with fine compasses on paper, ruled mathematically with squares whose sides measured 2 millims. In this way, as before said, we obtained a projection of the configuration of the cortex, the surface of which was thus already divided into squares of 4 square millims. in extent (see Plate 8, fig. 2), an arbitrary number being given to denote each square; these were successively stimulated, and the result recorded. Throughout this paper we shall refer, as before, to these units of localisation as "squares."

In every case the anæsthetic employed was ether, and the animal was killed before it recovered from the narcosis.

Method of Excitation.—The excitation, as before, was applied by fine platinum electrodes 2 millims. apart and furnished from the secondary coil of a Du Bois-Reymond inductorium served by a one-litre bichromate cell. The strength of the faradic current used was very weak, the distance of the secondary coil varying from 12 to a maximum of 9 centims. Momentary application of the electrodes was also resorted to for the detection of the initial contraction. For the complete development of any movement, and at the same time ensuring against the incidence of epilepsy, we found that usually 2 seconds was a sufficient duration for the stimulus.

Arrangement of the Results Obtained.—Even with every effort to obtain an animal of the same size and age and of the same variety, it is nevertheless impossible to avoid certain minute differences which render difficult the direct transference of the facts relating to one square in one animal to another square in another experiment.

We combined the results of our twenty experiments in the following manner, and from control observations subsequently carried out are sure that our procedure has not involved any serious error.

Mode of Notation.—As in each experiment we had made exact drawings of the sulci on the ruled paper (see fig. 2), it was easy to denominate the horizontal lines of squares in successive order from the fissure of Sylvius by capital letters, viz. :— A, B, C, D, E, &c. (see fig. 2), whereas the vertical rows were designated by italic letters a, b, c, d, e, &c., commencing opposite the præcentral sulcus and proceeding from front to back.

In this way any given square could be at once fixed and spoken of, for example, as Ad, Fe, &c. When thus in possession of a general classification the *average* position and inclination of the sulci could be discovered and pricked off.

By this means we constructed fig. 2, in which is correctly given the *average* position of the sulci and size of the gyri in all the cases observed by us.

Having therefore before us the average configuration of the area under discussion

and having denominated the squares subdividing it as above stated, the aggregation and collocation of the individual observations in each experiment became a very easy matter, since for any given portion of the surface explored the result of excitation in each animal was placed under the same heading.

To guard against the fallacy of producing a state of hyper-excitability in any one direction and to check our results containing the arrangement of representation, *i.e.*, whether vertical, or lateral, we moved the electrodes in different experiments sometimes vertically from square to square, at other times horizontally.

<div align="center">PRELIMINARY CONSIDERATIONS.</div>

Before giving a detailed account of the results obtained in these experiments, it will be advisable to say a few words about the phenomenon of bilateral representation.

Bilateral Representation.—In a previous paper* by us on the excitable fibres of the internal capsule the question of bilateral representation was discussed. The views of BROADBENT were there referred to, viz. :—that bilateral movement was effected by impulses passing from the sound hemisphere across commissural fibres postulated to exist between the lower (*i.e.*, bulbo-spinal) centres of the two sides ; we gave also a list of movements credited as being bilaterally represented. As this question is so important, we have reproduced this list (see Table), and desire to comment further upon it.

<div align="center">TABLE 1.—Credited as Bilateral.</div>

Movements of trunk muscles, *i.e.*, rectus abdominis ⎱	
Certain movements of tongue . . . . ⎰	Class I.
Conjugate deviation of eyes . . . . ⎰	Not bilaterally represented.
Turning of head . . . . ⎰	
Retraction of angle of mouth . . . . ⎱	
Pursing of lips	
Opening of eyelids	Class II.
Closing of eyelids	Imperfectly bilaterally represented.
Opening mouth , ...	
Elevation of soft palate . . . . ⎰	
Pouting of lips . . . . ⎱	Class III.
Mastication	Truly bilaterally represented.
Swallowing	
Adduction of vocal cords . . . . ⎰	

In our previous paper on the Internal Capsule we showed that movements of a special character and which we have placed in Class I. being elicited from only a definite part of the cortex, could not be regarded as being bilaterally represented, *i.e.*, as being represented in both hemispheres, and being evoked by excitation of either hemisphere. Consequently, for these movements, no substitution of function by the opposite side of the brain is conceivable.

* 'Phil. Trans.,' vol. 181 (1890), B, p. 73.

We desire now to say a few words respecting the present use of the term, bilateral movement, and also the term, bilaterally associated movement. As regards *bilateral movement* we believe we are right in stating that the general use of this term at the present time is partly the same as that of bilaterally associated movement, but this second term premises that there is an association between the representations in the nervous system of so-called bilaterally associated movements. We venture to think that the term bilateral movement should be strictly confined to its simplest meaning, viz., that in the execution of any definite muscular action or movement the muscles of both sides of the body are involved, though not necessarily to the same extent, or that the muscles acting are of the same name. We would urge that, physiologically speaking, the term bilateral movement can only mean simultaneous action of muscles on both sides of the body, and ought not to be used for the representation of those muscles in the nerve centres. We believe that a certain degree of laxity in the use of this expression has crept in by the employment of the word " associated." The term association connotes physiological co-operation and arrangement in the nervous system, *i.e.*, motor representation when the term is applied to movements. With this view the expression " bilaterally associated movement " has been frequently used to characterise the activity of the trunk muscles. We showed in our Internal Capsule paper that this was not justified by the facts, since the somatic or trunk muscles are as unilaterally represented, with the exceptions denoted in Table I., as are the limb muscles. This point has been treated of late by SHERRINGTON,[*] who has since shown that even the sphincter ani is unilaterally represented. Whatever may be the physical condition, *i.e.*, movements of different muscles for the performance of definite acts, there does not seem to be any ground for assuming, as has been so frequently done, that certain muscles, *e.g.*, those of the trunk, act in association, or that they are, therefore, bilaterally represented.

Apart from the special physiological interest attaching to this question of bilaterality, it is impossible for neurologists to correctly appreciate the pathology of epilepsy or the restoration of function when certain districts of the cerebral hemisphere have been destroyed until this question has been settled.

ANALYSIS OF RESULTS.

We have arranged the movements elicited by excitation of the region observed according to the following classification.

I. Orifacial Movements.

A. Face ...
- (a) Upper face .
  - (1) Eyelids . . . .
    - Opposite side . . . . { Closing
    - Bilateral . . . . . { Opening
  - (2) Eyes . . . . . Conjugate deviation to the opposite side . . Opposite side
  - (3) Angle of mouth . Elevation of . . . . Same side
- (b) Lips . . . .
  - (1) Upper lip . . . Inversion . . . . . { Opposite side
  - (2) Lower lip . . . Eversion . . . . . { Same side
    - { Bilateral
- (c) Lower face .
  - (1) Angle of mouth . Retraction of . . . { Opposite side
    - { Same side
    - { Bilateral
  - (2) Angle of mouth . Advancing of . . . { Opposite side
    - { Same side
    - { Bilateral
  - (3) Pursing of mouth
  - (4) Pouting of mouth
  - (5) Cheek
  - (6) Chin

B. Tongue . . . .
- (1) Movements towards the opposite side
- (2) Movements towards the same side
- (3) Bilateral movements

C. Lower jaw . . . .
- (1) Single movements . . . { Lateral
  - { Vertical
- (2) Rhythmical movements . . Mastication

D. Pharynx . . . .
- (1) Soft palate (elevation of) . { Opposite side
  - { Same side
  - { Bilateral
- (2) Swallowing

II. Movements otherwise than Orifacial.

A. Head.
B. Upper Limb.

We will now take the movements observed in the present research, seriatim, and give in detail first the squares in which they are represented, arranging these in the order of greatest frequency of occurrence of the movement. Under the same heading we shall treat of the individual points of importance and interest observed for each movement.

I. Orifacial Movements.

A. Face.

(a.) *Upper Face.*

   (1.) *Representation of the Movements of the Eyelids.*

      (a.) Closure . . . { Opposite eyelids.
                      { Eyelids of both sides.
      (b.) Opening . . . Eyelids of both sides.

(*a.*) *Closure of Opposite Eyelids* (fig. 3).—This movement, which possesses a well-defined focus of representation in the Monkey's cortex, corresponds to that seen in the cortex of the Dog (*vide* FRITSCH and HITZIG, FERRIER, LUCIANI, PANETH, and others).

Closure of the opposite eyelids was noted by us to occur at the following squares in the order of greatest frequency, as shown by the numbers :—

	5.	4.	3.	2.	1.			
Hj	Fj Gj	Fi Gi 1g	Fk Fl Ii	Ih	Ek El Em	Fm Gk Hl Hk	If Jf Ji Jj	Kf Kg Kh Ki

It will be understood that excitation of these points elicited only closure of the pair of eyelids of the opposite side to that of the hemisphere stimulated. It is very well shown to be unilateral in instantaneous (flash) photographs taken during excitation. We only once observed bilateral action, viz., closure of both pairs of eyelids at Gj.

There is in addition a very characteristic bilateral movement of the eyelids (*i.e.*, both pairs), popularly termed blinking, which requires description. This consists of a rhythmical closure, or opening of both pairs of eyelids. This is, of course, quite different to the movement just mentioned. We observed it to occur in three separate experiments upon excitation of the square Fj.

(*b.*) *Opening of the Eyelids of Both Sides.*—This movement we do not regard as being specially represented in the area under discussion (*i.e.*, posterior to the sulcus præcentralis, inferior and anterior to the lower end of the intraparietal sulcus), because, as has been shown by other investigators besides ourselves, the focus of this movement is situated in front of the præcentral sulcus. We noted it, however, to occur once at Ie in an animal in which the ascending frontal gyrus was exceptionally broad.

(2.) *Representation of the Movements of the Eyes.*

(*a.*) *Conjugate Deviation of the Eyeballs to the Opposite Side to that of Excitation.* —This well-known movement, the chief focus of which is situated in front of the præcentral sulcus, we elicited from the squares—

2.

Gl

These squares, it will be seen, are situated at the commencement of the supramarginal gyrus, or, as it is often improperly termed, the anterior limb of the angular

gyrus, wherein this movement has previously so often been observed to be represented (*vide* FERRIER, LUCIANI, SCHÄFER).

We observed no other movements of the eyeballs.

(3.) *Angle of Mouth, Elevation of.*

We subdivide the representation of the movement of elevation of the angle of the mouth under the following headings :—

(*a.*) Elevation of the opposite angle of the mouth.

(*b.*) Elevation of the angle of the same side.

(*c.*) Elevation of both the angles of the mouth.

(*a.*) *Elevation of the Angle of the Opposite Side* (fig. 4).—This, by far the most frequent movement of elevation, was observed at the following large number of squares :—

7.	6.	5.	4.		3.	2.	1.		
G*g*	E*j*	E*k*	D*k*	H*f*	E*g*	D*i*	C*k*	E*a*	J*f*
G*h*	G*f*	F*y*	E*i*	I*h*	F*f*	D*j*	C*i*	E*f*	J*y*
	G*i*	H*g*	F*k*		F*i*	E*h*	C*j*	F*d*	J*h*
	H*h*		F*j*		G*j*	E*l*	C*k*	G*d*	
	I*g*		F*h*		H*j*	F*l*	D*e*	H*k*	
					I*f*	G*a*	D*l*	I*i*	

As is seen in fig. 4 the focus of greatest representation is at G*g*, G*h*.

(*b.*) *Elevation of the Angle of the Same Side.*—The representation of this movement, which from *à priori* considerations we did not anticipate finding, was observed in single instances at squares :—

$$A f \qquad B j \qquad C j \qquad D i \qquad F h.$$

(*c.*) *Elevation of both the Angles of the Mouth.*—As a corollary to the facts just related, the actual observation of bilateral representation of this movement of elevation of the angle of the mouth was seen on two single occasions, viz., at squares—

$$C l \qquad C e.$$

This bilaterality is therefore very rare.

(*b.*) *Lips.*

In describing the movements of the lips we have observed occasionally such unilaterality of representation as to lead us to regard the lips, *e.g.*, the upper and the lower as divisible into two halves, viz., the right and the left. We have, therefore, subdivided the lips into four regions, a right and left upper and lower lip. We thus classify the movements noted, according as to whether they affect the upper or lower

lip individually, and further whether it is of the opposite or of the same side, or whether both sides move at once.   The movements observed are as follows :—

(*a.*) Elevation of the upper lip of the opposite side.

(*b.*) Depression of the lower lip of the opposite side.

(*c.*) Inversion of the lower lip of the opposite side.

(*d.*) Inversion of both lips of the same side.

(*e.*) Inversion of both lips of both sides, but especially of the opposite side.

(*f.*) Inversion of both lips.

(*a.*) *Elevation of the Upper Lip of the Opposite Side.*—This is a rare movement of remarkably unilateral character which differs from simple elevation of the angle of the mouth in that the ala of the nose is not moved.   It was observed to occur only once at squares B*j*, C*f*, and C*g*.

(*b.*) *Depression of the Lower Lip of the Opposite Side* (fig. 5).—This movement is represented at the following squares :—

3.	2.	1.	
F*g*	F*e*	F*h*	I*i*
I*g*	G*g*	G*d*	J*f*
	H*g*	G*e*	J*g*
	I*f*	G*f*	
	I*h*		

(*c.*) *Inversion of the Opposite Lower Lip.*—This, as a solitary movement, occurred only once at G*l*.

(*d.*) *Inversion of both Lips of the Same Side.*—This occurred twice at E*h*.

(*e.*) *Inversion of both Lips of Both Sides, but especially the Halves of the Opposite Side.*—In this case the whole length of the upper and lower lips was inverted, but the opposite halves were affected notably more than those of the same side. The movement was found at squares—

		1.		
F*e*	A*f*	E*c*	F*f*	H*e*
F*j*		E*d*	·F*k*	H*f*
G*i*		E*e*	G*e*	H*j*
G*j*		E*i*		H*k*
G*k*		E*j*		H*l*
I*f*		E*k*		

(*f.*) *Inversion of Upper and Lower Lip of Both Sides.*—This movement is similar to the one just described, except that it was completely bilateral, no difference being found between the two sides. It occurred once at A*g*, B*f*, B*g*, B*h*, B*i*, B*j*, D*i*, D*k*, E*h*,

(*c.*) *Lower Face.*

We now come to the movements of the lower part of the face and commence with those of the angle of the mouth. These may be suitably arranged as follows :—

(*a.*) Retraction of the angle of the Mouth of the opposite side.

(*b.*) ,,	,,	same side.
(*c.*) ,,	both sides.	
(*d.*) Advancing	the opposite side.	
(*e.*) ,,	,,	same side alone.
(*f.*) ,,	,,	both sides.
(*g.*) ,,	the same side together with the cheek.	
(*h.*) Depression		

(*a.*) *Retraction of the Angle of the Mouth of the Opposite Side* (Plate 8, fig. 6).— This, with that of elevation of the angle of the mouth, is the most important movement of the face, and has long been recognized to be so. In the movement of retraction, the angle of the mouth is drawn horizontally backwards without necessarily parting the lips. It occurred at squares—

12.	9.	8.	7.	6.	5.	4.	3.	2.	1.
Dj	Ej Hg	Eh Hh	Eg Ei Fi Fj Gg	Dh Fg Hf	Cj Di Gf Hj	Df Fe Ff Fh Gh Gj Ie Ig	Ch Ck Dl Eh Fk Gi Gk Gl Ih	Bg Bi Bj Dh Ee Ef Fm Hl Jf Jg	Bh Cf Cg Ch Dg Ed El Fl Gd Ge Hk Jh

(*b.*) *Retraction of the Angle of the Mouth of the Same Side.*—Particular notice must be taken of this movement as it occurs on the *same* side as that of the hemisphere excited. It was found in squares—

2.	1.		
Af Bf Eg	Ag Ai	Bi Ob Df	Eg Gh

Its relative infrequency is very obvious.

(*c.*) *Retraction of the Angle of the Mouth of Both Sides* (fig. 7).—This truly bilateral movement was seen at squares—

3.	2.	1.		
C*j*	C*i*	C*g*	D*g*	D*h*
C*k*	E*f*	C*h*	D*i*	D*l*
	E*g*	D*e*	D*j*	E*f*

The next kind of movement which we noticed to affect the angle of the mouth was in the opposite direction to that just mentioned of retraction, and may properly be described as advancing. In this the angle of the mouth is drawn or pushed forward, and the movement may occur on either the side opposite to that of the hemisphere excited, or on the same side, or bilaterally, *i.e.*, both angles of the mouth are advanced simultaneously, and, as will be subsequently seen, also accompanied by movement of the cheek.

(*d.*) *Advancing of the Angle of the Mouth of the Opposite Side.*—This, the crossed unilateral action as generally understood, we only noted to occur in single instances at

<div align="center">D<i>h</i>   G<i>e</i>   G<i>g</i>   G<i>h</i>   H<i>f</i>   H<i>g</i>.</div>

(*e.*) *Advancing of the Angle of the Mouth of the Same Side Alone.*—This rare movement was observed only once at D*h*.

(*f.*) *Advancing of the Angle of the Mouth of Both Sides.*—This, which is a well-marked bilateral movement, is in our opinion the forerunner to the important movements of pouting and pursing of the mouth. We observed it repeatedly at squares, viz.:-

3.	2.	1.			
F*g*	D*h*	B*e*	D*d*	D*f*	D*k*
	G*f*	C*f*	D*e*	D*g*	G*h*

(*g.*) *Advancing of the Angle of the Mouth of the Same Side together with the Cheek.* This combined action of the forward movement of the angle of the mouth and cheek was only observed twice at C*h*, and must be looked upon as an exceptional development of the advancing of the angle of the mouth.

(*h.*) *Depression of the Angle of the Mouth (i.e., Platysma Action).*—We only observed this movement to occur twice, viz., at centres C*g* and C*h*.

(3) *and* (4). *Pursing and Pouting.**

There remain for consideration certain combined actions of the lips and orifice of the

<div align="center">* ' Phil. Trans.,' B, 1890, p. 136.</div>

mouth which are easily recognized under the quasi-popular expressions of pursing and pouting, but which cannot be described sufficiently accurately except by long periphrasis. To avoid this we employ the former expressions under the following meanings.

Pursing.—By this term, as we have already described in our paper on the motor cortex of the Orang,[*] we mean the drawing together of the lips, together with slight protrusion, both parts of the orbicularis being contracted, whilst in *Pouting* the lips are strongly protruded, and at the same time they are much everted.

We may subdivide this subject in the following manner :—

(*a.*) Pursing of the opposite side of the mouth.
(*b.*) Pursing of both sides of the mouth (bilateral).
(*c.*) Pouting of the opposite side of the mouth.
(*d.*) Pouting of the same side of the mouth.
(*e.*) Pouting of both sides of the mouth.

(*a.*) Pursing of the opposite side of the mouth was only observed once at the squares E*c*, E*d*, E*e*, I*g*.

(*b.*) Pursing of both sides of the mouth occurred at—

2.	1.		
E*g*	E*h*	F*h*	G*f*
F*g*	F*e*	F*h*	H*e*
H*f*	F*f*	G*e*	H*g*

(*c.*) Pouting of the opposite side occurred at·

2.

D*i*   D*j*   E*e*   E*c*   F*d*

(*d.*) Pouting of the same side of the mouth occurred only once at D*h*
(*e.*) Pouting of both sides of the mouth was seen at—

3.	2.		1.				
F*h*	B*e*	E*f*	A*d*	B*f*	C*h*	F*e*	G*h*
	C*e*	F*f*	B*b*	C*b*	E*g*	F*g*	
	D*i*	G*g*	B*d*	C*c*	E*h*	G*f*	

(5.) *Movements of the Cheek.*

In close relation with the movements of the angle of the mouth are those of the cheek, which may be divided into the two following groups :—

　(*a.*) Flattening of the cheek on the same side.

　(*b.*) Flattening of both cheeks.

(*a.*) *Flattening of the Cheek on the Same Side* (fig. 8).—This movement was found at the following squares :—

4.	3.	2.	1.	
D*g* C*f*	D*h*	B*y* C*y* E*h* G*h* E*f* E*g*	B*d* B*f* D*f*	E*d* G*y*

And it was seen to be almost invariably associated with a special movement of the tongue, viz.: rolling over of the dorsum to the cheek of the same side (p. 55), so that the cheek moved to meet the dorsum of the tongue, and would thus force, and keep the bolus of food between the molar teeth.

It is interesting to note that this movement occurred as a unilateral action and only on the same side as that of the cortex stimulated.

(*b.*) Bilateral flattening of the cheeks occurred only as a single instance at E*h*. It is, therefore, very exceptional.

(6.) *Movements of the Chin.*

The movements of the chin are the last we have to note, of those observed in the lower face.

They may be considered under the heads of—

　(*a.*) Elevation of the chin on the opposite side.

　(*b.*) Elevation of the chin on the same side.

　(*c.*) Bilateral elevation of the chin.

(*a.*) *Elevation of the Chin on the Opposite Side.*—This is by far the commonest movement of the chin met with, and is probably caused by the levator menti muscle. It occurred at—

2.	1.			
D*i* E*j* E*k*	C*h* C*i* C*j*	D*g* D*h* D*j*	D*k* E*i* E*l*	F*h* F*k*

(*b.*) *Elevation of the Chin on the Same Side.*—This was very rare and occurred as a single observation at E*g*.

(*c.*) *Bilateral Elevation of the Chin.*—This occurred only once at squares C*h*, E*h*.

B. *Movements of the Tongue* (Plate 9, fig. 9).

The question of the manner in which the various movements of the tongue should be grouped was alluded to in the consideration of bilateral representation on p. 44. We have decided to divide the movements of the tongue into—

    1. Movements bilaterally represented.

    2. Movements not bilaterally represented.

They may therefore be arranged in the following order :—

    1. Movements bilaterally represented.
        (1.) Tongue protruded straight.
        (2.) Tongue retracted straight.
    2. Movements not bilaterally represented.
        (1.) Tongue protruded, tip to the opposite side.
        (2.) Tongue protruded to the same side.
        (3.) Tongue retracted on the same side.
        (4.) Tongue rolled over with the dorsum to the cheek of the same side.

1. *Movements Bilaterally Represented.*

(1.) *Tongue Protruded Straight* (Plate 9, fig. 10).—This simple bilaterally represented movement, *i.e.*, which can be evoked equally well from the cortex of either hemisphere, was found at the following squares :—

6.	5.	4.	3.	2.	1.
A*e*	B*e*	A*b*	A*c*	B*g*	A*b*
B*f*	C*d*		A*d*	C*e*	B*d*
C*e*			A*f*	C*g*	B*e*
			A*g*	C*j*	B*d*
			C*f*	D*g*	C*b*
			D*d*		C*i*
			D*e*		C*k*
					D*e*
					D*f*
					E*i*
					F*i*

In this movement the tongue is protruded quite flatly and without any deviation of the tip to one side, and without any heaping up posteriorly.

(2.) *Tongue Retracted Straight.*—This was less widely represented than the former, and was found at—

3.	2.	1.		
Bh	Bg	Ce	De	Gh
Ce	Bi	Ch	Dh	
		Cj	Dj	

2. *Movements not Bilaterally Represented.*

(1.) *Tongue Protruded with the Tip to the Opposite Side* (fig. 11).—This is the movement upon which so much stress has been laid in clinical observation, and the real nature of which will further on be more minutely discussed. It was found to be represented at squares—

4.	3.	2.					1.					
Df	Bi	Ai	Ck	Dk	Ek	Gg	Bb	Cg	Eo	Ff	Gj	Ih
Eg	Ci	Be	De	Ee	Fi	Gh	Bc	Ch	Ed	Fh	Hf	Je
	Dj	Bg	Dg	Eh	Fg	He	Bd	Dd	El	Ej	Ie	Jf
	Ei	Cf	Di	Ej	Ge	Hh	Bh	Dh	Fd	Gd	If	Jg
	Hg	Cj					Ce	Dl	Fe	Gf	Ig	

(2.) *Tongue Protruded with the Tip to the Same Side.*—This movement, in marked contrast to the former one, was obtained three times at only one square, Af.

(3.) *Tongue Retracted on the Same Side.*—This movement, which is very slightly represented, was found once at Gg.

(4.) *Tongue Rolled over so that the Dorsum comes into contact with the Cheek of the Same Side* (fig. 12, cf. fig. 8).—This movement is one in which there is no protrusion of the organ, but in which it is so rotated on its longitudinal axis that the dorsum of the tongue is closely applied to the mucous membrane of the cheek of the same side as that of the cortex stimulated. As previously stated, the cheek of the same side is flattened to meet the convexity of the approaching tongue. It occurred at—

5.	4.	3.	2.	1.		
Bg	Cg	Df	Ah	Bf	De	Ek
	Ch	Dg	Bh	Cb	Eg	Fg
	Dh		Ce	Cf	Eh	
			Bf	Ci	Ei	
			Fh	Cj	Ej	

Consideration of the foregoing Facts with especial Reference to Movements of the Individual Halves of the Tongue after Separation along the Median Plane.

We have already described the movements of the tongue as a whole, and we have alluded to the questions of unilaterality and bilaterality of movement of this organ. A new and unexpected light was thrown on this subject by the following attempt to differentiate the complex movements of the entire tongue, and to determine what share is taken by each half in the production of any given action.

It occurred to us that much might be learnt by regarding the tongue, at least for the longitudinal muscles, as constructed of two similar halves; further, that these might be separated from each other by a vertical incision in the middle line, and this without injury to the nerve supply of each half.

Of course we recognize that this procedure involves the median* division of the septal origin of the transverse muscular fibres and also the division of any hypothetical nerve fibres crossing the raphe, but this does not invalidate the results we obtained, and which show most definitely that the movements of both halves of the tongue are represented in the hemisphere of each side respectively. For illustration of our meaning, and for the better comprehension of the facts about to be related, we may compare the question of this bilateral representation of the movements of the respective halves of the tongue with those of the conjugate movements of the eyes, and it will be seen presently that the common, and in hemiplegia previously supposed unilateral, movement of the tongue is really a conjugate movement.

The operation of separation was performed by dividing the tongue along the median plane from the hyoid bone and root of the epiglottis forward to the genial tubercles. As a rule there was exceedingly little hæmorrhage, and this was easily arrested by pressure. The reflex movements of the tongue were as complete as before, showing that the functions of the organ were not interfered with.

We will now take the movements of the tongue as a whole (see p. 54), and discuss in the light obtained by this method how far the change of form of the whole organ is dependent on each of the halves respectively; we will therefore take the movements in the same order in which they have just been considered.

Movements Bilaterally Represented.

(1.) *and* (2.) *Advancing and Retraction of the Tongue.*—We observed that in these movements executed symmetrically forwards or backwards, each half of the tongue performed exactly the same evolution, and in no case could preponderance of action be shown to take place on either side. It follows therefore that these movements are truly bilaterally represented.

* Practically the line of separation frequently passed close to the side of the septum, this, of course, not interfering with the power of the transverse fibres to contract, and so narrow the half of the tongue.

Movements not Bilaterally Represented.

(1.) *Protrusion of the Tongue to the opposite side.*—From the time of ABERCROMBIE*
it has been noticed in clinical medicine that in hemiplegia the tongue was protruded
towards the paralyzed side, *i.e.*, opposite to the lesion, and this was assumed to be
due to the genio-hyo-glossus muscle of the healthy side (*i.e.*, the same as the lesion)
pressing the tongue towards the paralyzed side, *i.e.*, away from the lesion.

In CARPENTER's Physiology† it is stated that the deviation is due to the want of
action of the lingual muscles of the paralyzed side, the tip being directed by the
muscles of the other (*i.e.*, healthy) side, " which will not act in a straight direction
when not antagonized by their follows."

In short, it is evident that a general belief exists to the effect that if one side of
the tongue is being actively protruded, the other half remaining passive the resulting
position of the tongue is a deviation towards the paralyzed side owing to the passive
flaccid condition of the non-acting muscles.   The first point which we think requires
to be definitely ascertained in treating this subject is the determination as to whether
excitation of *one* hypo-glossal nerve produces any movement of the tongue across the
median plane, as is evidently generally supposed.   We ourselves having foreseen the
complex problems involved in the movements of this part especially re-investigated,
by excitation experiments at the base of the skull, the functions of several cranial
nerves separated from the medulla.   We found ('Roy. Soc. Proc.,' vol. 44, June 7,
1888) that excitation of the hypo-glossal nerve of one side produced in the whole
tongue " flattening posteriorly on the same side and the tip protruded also on
the same side."   The purely unilateral character of the movement was further
established by experiments in which the tongue was divided longitudinally to the
hyoid bone when the movements were seen to be entirely confined to the side
stimulated.

It will therefore be seen that stimulation of the hypo-glossal nerve of one side does
not protrude the tongue towards the opposite side.

We will now proceed to describe what happens to the individual parts of the
tongue when in the left cortex the squares for the representation of protrusion of the
tongue to the opposite side are excited.   Instead of the right half of the tongue
remaining passive as has always been presupposed by anatomists and clinicians,
we discovered that when the left cortex was stimulated the following remarkable
conjugate movement occurred :—the left half of the tongue was advanced beyond the
teeth, whereas the right half was actively retracted into the mouth.   It is perfectly
obvious therefore, that this movement of protrusion of the tongue to the opposite

* 'Pathological and Practical Researches on Diseases of the Brain and Spinal Cord,' Edinburgh, 1834,
p. 271.

† CARPENTER's 'Physiology' edited by POWER, 7th edition, 1869.   Compare also ROSS' 'Treatise on
Diseases of the Nervous System,' 1883, and GOWER's 'Diseases of the Nervous System,' 1888.

side from stimulation of the cortex is exactly comparable to the conjugate movement of the eyes to the opposite side when the same hemisphere is excited. In this way the right half of the tongue would correspond to the right external rectus, and the left half of the tongue to the left internal rectus. In each case we have the combination of two opposite movements for the production of one resultant action. The opposite character of the movements of the two halves of the tongue was emphasized in a very remarkable degree when there was epileptic disturbance of the cortical focus in question. In the clonic spasm of the epileptic fit the two halves performed remarkable "see-saw" contractions, that of the same side being shot forwards at the same time as the opposite half was retracted. On several occasions we observed that retraction of the opposite side not only began before the protrusion of the same side, but actually in a few instances it occurred alone, thus showing that it is probably the more important movement of the two. We are now in a position to examine into the condition of the tongue as observed in hemiplegia. Unfortunately the clinical examination of the tongue has hitherto not been conducted so thoroughly as its importance warrants; and in fact, clinical observation is usually limited to noticing whether the tongue is deviated to one side when protruded, without further exploration of other movements. This is doubtless due to the fact that the movements of the tongue generally have hitherto not been completely ascertained. Although this makes the task of explanation very difficult and for the present impossible, we take leave to point out that the accepted views are plainly contrary to fact and will have to be altered.

(2.) *Rolling over with the Dorsum directed towards the Check of the Same Side.*— This movement we found to be distinctly compound in character, both separated halves of the tongue rolling to the same side. In the execution of this movement the mucous membrane of each half of the tongue was turned so as to be directed towards the left cheek, as a result the cut surfaces glided upon each other so that the two halves were arranged *en échelon* in place of being in line; the cut surface of the same side being directed upwards and that of the opposite side downwards.

Each half of the tongue therefore executes a rotation around its longitudinal axis.

Vertical Arrangement of the Representation of the Movements of the Tongue.

Reference to the diagrams (Plate 9, fig. 9, &c.) will show that a very definite differentiation of the representation of the tongue movements can be made as follows. In the upper part of the area, *i.e.*, just below a line drawn between the upper end of the præcentral vertical stem and the genu of the fissure of ROLANDO, the action of protrusion of the tongue with the tip directed to the opposite side is elicited on excitation. On the contrary, the movement of retraction is represented at the lowest portion of the area, *i.e.*, around the lower end of the fissure of ROLANDO. Intermediately, *i.e.*, opposite the upper end of the sulcus transversus frontalis inferior (see &c.) we obtain the movement of rotation of the tongue towards the same side.

C. *Movements of the Lower Jaw.*

The movements of the lower jaw may be divided into :—

1. Single movements.
   - (1.) Opening the mouth straight.
   - (2.) Opening of the mouth with the lower jaw directed towards the same side.
   - (3.) Opening of the mouth with the lower jaw directed towards the opposite side.
   - (4.) Shutting the mouth.
2. Rhythmical.
   - (1.) Mastication.

1. *Single Movements.*

By the term " opening the mouth " we understand that the lower jaw is depressed. This movement we have observed to occur without necessarily separating the lips, which additional passive action, however, nearly always happens concurrently. We therefore consider these two cases.

We look upon the movement as being accomplished by the muscles of both sides, but we have no evidence to show whether they act concurrently and whether the movement, if it does not deviate from the middle line, is capable of being produced by the muscles of one side only. It is known that if one external pterygoid muscle act alone, the jaw on being depressed is carried towards the opposite side, and we therefore assume that this occurs when the jaw deviates towards one side or the other.

(1.) *Opening the Mouth Straight* (fig. 13).—In this movement the jaw is depressed exactly straight along the line between the two central incisors. It was found at—

10.	9.	8.	7.	6.	5.	4.	3.	2.	1.
	Af	Cg	Bg	As	Cj	Cd	Ab	Ac	Bc
	Ag	Ci	Bi	Ah		Ck	Ad	Bd	Cl
	Bf		Cc	Bs		De	Ai	Dc	Dj
			Ch	Bh		Eg	Bd	Di	Dk
				Cf			Bj	Eh	Ef
				Df			Cc	Ei	Ej
				Dh			Dd	Ek	Hg
							Gg	Fh	Hl
								Gh	

(2.) *Opening the Mouth with the Lower Jaw Carried to the Same Side* (fig. 14).— In this movement, with stimulation of the left hemisphere, the lower jaw as it descended moved markedly towards the left side; this we take to be due to the fact that either the right pterygoid muscle alone was in action, or that it overpowered that of the left side. While as just seen the commonest movement of depressing the lower jaw in opening the mouth is the bilateral or straight movement, where any

deviation was noticed, it was this one of the lower jaw passing to the same side as that of the cortex excited. This might have been anticipated from what has been stated, as it is obviously an instance of the ordinary crossed effect of stimulation. This movement occurred at—

4.		2.		1.	
B*g*	C*g*	A*e*	D*h*	A*d*	B*h*
D*g*		A*f*	E*h*	B*e*	C*h*
		B*d*	E*j*	B*e*	C*f*
		C*d*		B*j*	C*h*

(3.) *Opening of the Mouth with the Lower Jaw carried to the Opposite Side.*—This, which is the converse of that described, and was very rarely observed, is conceivably due to the action of the external pterygoid muscle of the same side, and was obtained only once at two squares, A*d*, A*e*.

On looking at the previous Table it will be seen that the movement of the jaw towards the *same* side was also obtained twice at A*e* and once at A*d*, thus showing that there are squares where the differentiation of the movement of the lower jaw towards the opposite side was incomplete, its production being due, therefore, more or less to an accidental causation.

(4.) *Shutting of the Mouth.*—This well recognized movement of closing the mouth was, to our surprise, obtained at only one square, viz., D*h*. By this movement we mean simply raising up the depressed lower jaw, without any further action, and the very small representation of this deliberate act is in marked contrast to that of the rhythmical movements to be next described.

2. *Rhythmical Movements.*

(1) *Movements of Mastication* (fig. 15).—By the term "mastication" we mean that on the application of the electrodes to the cortex, the lower jaw begins to execute a series of rhythmical movements of grinding of the teeth. To describe the movement in detail:—the mouth is opened, the lower jaw depressed and carried towards the same side, and then the mouth is closed by raising the lower jaw; directly the teeth are separated the tongue is advanced, and it is retracted immediately before the teeth come together again; this latter movement of the tongue is thus dependent on and coincident with the action of the lower jaw. This complex act of mastication begins immediately the electrodes are applied, and continues until the electrodes are removed, when it ceases. The movement is what would generally be termed that of mastication of a very pronounced type, and as such it differs somewhat from the description given by Professor Ferrier, in that he does not seem to have observed the grinding movements of the lower jaw. The most important point concerning it is

that it is an instance of rhythmical discharge of the cortex evoked by a constant stimulus, and lasts only during the duration of the excitation. So far as we know, it is the only truly rhythmical action following on stimulation of the cortex.*

With a view to seeing what this rhythmical rate might be we observed the time of it in seventeen cases, and found it to be on an average 1·35 movements per second, the most rapid rate observed being nine times in five seconds, and the slowest being one in one second, and each of these extreme cases was noted three times.

This compound act was observed at—

			3.	2.	1.
B*c*	B*d*		A*e*	A*g*	B*b*
			A*f*	C*g*	C*b*
		C*e*	B*g*	D*e*	C*i*
			C*e*	D*g*	C*j*
			C*h*	E*f*	D*h*
			D*e*	E*g*	E*d*
			D*f*		E*h*
					F*e*
					F*f*
					F*h*

Grinding of the Teeth.—On three occasions the teeth were observed to grind during the act of mastication, and in two of these the movement of the jaw was traced and found to pass from the opposite side to the same side. The squares where it was observed were A*d*, A*e*, A*f*.

Movements of the Pharynx.

In observing these movements, we kept the mouth forcibly opened, and recorded, as far as possible, the movement of, 1, the soft palate; and 2, that of swallowing.

1. *Movements of the Soft Palate* (fig. 16).

We may arrange the movements of the soft palate as follows :—

(1.) Elevation of the soft palate on the opposite side.

(2.) Bilateral elevation of the soft palate.

(3.) Bilateral elevation of the soft palate, commencing with elevation of the opposite side, followed later by that of the same side.

(1.) *Elevation of the Soft Palate on the Opposite Side.*—This movement of the soft palate, when viewed in the manner described, is quite unmistakable when it occurs; but it has to be carefully distinguished from the irregular movements of the arches of the fauces, when these are drawn forward by the tongue and by the movements of this organ itself. The movement was only observed once, at B*d*; and at the same

* Compare as an imperfect rhythm the act of blinking, see p. 47.

place slight opening of the mouth and protrusion of the tongue to the opposite side were noted.

(2.) *Bilateral Elevation of the Soft Palate.*—This movement, which is the commonly understood effect—viz., the raising of the whole soft palate—was seen only four times, viz., once at squares Ad, Ae, Bd, Cd. Of these at Ad, Bd, Cd, this was the only movement obtained; while at Ae there was also the movement of mastication.

The foregoing refers to experiments in which the observation of the soft palate was made at the end of the exploration. It occurred to us that possibly the paucity of these results was due to exhaustion of the cortex. We therefore devoted two experiments to examine this point first, with the effect that we obtained very definite localisation of the palate movements.

(1.) ELEVATION of the Soft Palate on the opposite side.

1.

Bf	Ad
Cf	An
	Af
	Bd
	Bn
	Cd
	Cn

(2.) BILATERAL Elevation of the Soft Palate

1.

Ae
Be
Be
Ce

(3.) BILATERAL Elevation commencing with elevation of the opposite side, followed later by that of the same side.

1.

Bd
Cd

These further observations showed clearly the real degree of representation of the palate, and in addition, what we did not anticipate, namely, a considerable degree of unilaterality of representation.

2. *Movement of Swallowing.*

This movement was observed once at four squares, viz. :—

Ad, Ce, Cj, Df.

The paucity of representation is perhaps accounted for by the movement of swallowing in the Monkey being more automatic than is generally considered to be the case, but it is possible that the effect of exposure may influence the result.

In relation to this question, the recent observations of GOLTZ,* in which he successfully removed both cerebral hemispheres in the Dog, leaving only the cerebellum, pons, bulb, and posterior corpora quadrigemina intact (the anterior partially so), and kept the animal alive for many months, must be specially mentioned. The animal had to have milk poured down its throat through a tube, because the act of swallowing was so far interfered with that fluid almost always escaped into the larynx. In two months after the operation, it would, when its nose was touched with a piece of meat, seize it, masticate it, and swallow it without any assistance. There seems, therefore, on the whole, to be no real reason why in the Monkey the cortical representation of swallowing should be more extensive than such as we find it to be.

II. MOVEMENTS OTHERWISE THAN ORIFACIAL.

In exciting the area under examination, since we were devoting ourselves especially to the orifacial movements, we only noted the concurrence of movements of other parts of the body when those appeared to us anomalous, and in order to satisfy ourselves of the extent of such extra movements, and to control the observations which we made in 1886, and also to ascertain to what extent the orifacial area overlapped the neighbouring areas.

The movements thus noted comprised those of the head and of the upper limb.

A. *Movements of the Head.*

Since the region which we have explored in the present research is in direct continuity with the supramarginal gyrus, and this with the anterior limit of the angular gyrus, it was to be anticipated that we should observe movements of the head and eyes to the opposite side, inasmuch as FERRIER, SCHÄFER, and others, observed these movements to occur from excitation of this region. The movements of the head which we noticed were (1) the turning of the head to the opposite side, and (2) retraction of the head.

* 'Archiv für d. Gesammte Physiologie' (PFLÜGER), vol. 51, p. 570, 1892.

(1.) *Turning of the Head to the Opposite Side.*—This movement, where the head is turned away from the side of the cortex stimulated, was found at the following squares :—

4.	3.	2.		1.			
D*l* D*m*	C*l*	E*j* G*k* F*k* 1*f* F*l* F*m*		B*j* E*k* G*l* J*f* C*k* E*m* H*m* J*h* D*j* F*i* 1*a* 1*g* 1*h*			

The representation of this act is, therefore, round the fringe of the orificial representation, especially at the posterior part near to the supramarginal gyrus, and also at the upper part of the region where it borders on the area representative of this movement in front of the præcentral sulcus.

(2.) *Retraction of the Head.*—Similarly the rarer movement of retraction of the head was noted to occur once at each of the following squares :—C*j*, C*k*, D*h*, D*i*, E*j*, F*h*, F*i*, F*k*, I*g*.

B. *Movements of the Upper Limbs.*

Although we noted the various movements of the segments of the upper limb we did not record the occurrence of the same in the present work with detail, but we have made a considerable series of observations to re-investigate the question of overlapping, and the extent of border centres, and recorded also certain anomalies of representation. In the present instance we have specially noted the lowest points at which movements of the upper limb could be elicited. These when arranged give the following results, commencing with the thumb and fingers, which according to our observations, both in 1886 and in the recent experiments, are the lowest segments represented.

	In front of Rolando.						Behind Rolando, anomalous.			
Thumb	D*d*	D*e*	E*f*	E*g*	G*h*		H*j*	C*j*	C*k*	
Fingers	D*c*	D*d*	D*e*	E*f*	F*g*	G*h*	G*j*	E*k*	D*l*	D*m*

In contrast to these segments we give the lower limits of the representation of the elbow and shoulder, as found in the present experiments.

	In front of Rolando.		Behind Rolando, anomalous.			
Elbow .		E*d*	E*i*	E*j*	E*k*	E*l*
Shoulder	I*f*	I*i*	C*k*	C*l*	E*m*	

These lower borders of the upper limb area of representation correspond absolutely closely to the levels given in the paper in 'Philosophical Transactions,' 1887.

In addition, however, we observed, to our surprise, that we occasionally obtained from the cortex of the foot of the ascending parietal gyrus movements of the upper limb, and not merely of the lowest segments, but also of the shoulder. Of fifteen experiments we noted at C*k* that the thumb was flexed twice, the fingers flexed twice in the interosseal position, and the shoulder adducted once at C*l*. The great rarity of this representation of the shoulder so low down on the outer or convex surface of the hemisphere, shows that it is quite exceptional, and no explanation of its occurrence in the evolution of the function of this part presents itself to us.

TABLE I.—Giving Absolute Number of Movements noted at any given Square, arranged in Order according to Frequency of Occurrence and not according to their Relative Sequence. See Table II. of "Marches."

T. = tongue.
M. = mouth.
∠ = angle of mouth.
U.L. = upper lip.
H.l. = head.
F. = fingers.

Th. = thumb.
Sh. = shoulder.
W. = wrist.
Elb. = elbow.
S.P. = soft palate.

Abbreviations to Table I.

retr. = retraction.
∠ = angle of mouth.
opp. = opposite.
str. = straight.
protr. = protrude.
add. = adduct.

eler. = elevate.
inteross. = interosseal.
mastic. = mastication.
adv. = advance.
dors. = dorsum.
abd. = abduct.

flat. = flatten.
pout. = pouting.
bilat. = bilateral.
flex. = flexion.
ext. = extension.
sup. = supinate.

pron. = pronate.
rot. = rotate.

Square.					Number of observations.	Nil.
A.b.	Mastic., 4.	T. protr. str., 4.	M. open, 3.		15	10
A.c.	Mastic., 3.	T. protr. str., 3.	M. open, 2.		15	11
A.d.	Mastic., 4.	T. protr. str., 3. M. open, 3.	M. open, jaw to same side, l. M. open, jaw to opp. side, l. S.P. elevated bilaterally, l.	Pouting bilat., l. Grinding teeth, l. Swallowing, l.	15	5
A.e.	M. open, 6.	T. protr. str., 6.	Mastic., 5.	M. open, jaw to same side, 2.	M. open, jaw to opp. side, l. S.P. elevated bilat., l. Teeth grind from opp. to same side, l.	15	4
A.f.	M. open, 9.	T. protr. str., 3. T. protr. to same side, 3.	Mastic., 3.	M. open, jaw to same side, 2. M. ∠, same side retract., 2.	M. ∠, same side eler.., l. Grind teeth, l. Lips invert. (espec. opp. side), l.	15	4
A.g.	M. open, 9.	T. protr. str., 3.	Mastic., 2.	M. ∠, same side re[tract], l. Lips invert., l.		15	4
A.h.	M. open, 6.	T. rolled to same side, dors. to cheek, 2.		T. protr. str., l.		15	9
A.i.	M. open, 3.	T. protr. tip to opp. side, 2.	M. ∠, same side re[tract]			15	10

LE I. (contin

Square.						Number of observations.	Nil.
B.b.	M. open, 2.	Mastic., 1. T. protr. str., 1. T. protr. tip to opp. side, 1. Pouting, 1.				15	13
B.c.	Mastic., 5.	Pouting, 2.	M. open, jaw to same side, 1. M. open, 1. T. protr. str., 1. T. tip to opp. side, 1.			15	8
B.d.	Mastic., 7.	M. open str., 3.	M. open, jaw to same side, 2.	T. protr. str., 1. T. protr. tip to opp. side, 1. S.P. elev. bilat., 1. S.P. elev. opp. side, 1.	Cheek flattened, 1. Pouting, 1.	15	4
B.e.	Mastic., 8.	M. open str., 6.	T. protr. str., 5.	T. tip to opp. side, 2.	M. open, jaw same side, 1. M. ∠ adv., 1.	15	3
B.f.	M. open str., 9.	T. protr. str., 6.	Mastic., 4.	M. ∠ (same) retract., 2.	M. open, jaw same side, 1. T. rolled dors. to same cheek, 1. Cheek flattened, 1. Pouting, 1. Lips inverted, 1.	15	4
B.g.	M. open str., 7.	T. dors. rolled to same cheek, 5.	M. open, jaw to same side, 4.	Mastic., 3.	T. tip to opp. side, 2. Lips inverted, 1. T. protr. str., 2. T. retracted, 2. M. ∠ opp. retr., 2. M. ∠ same retr., 2. Cheek flattened, 2.	15	2

T.   ...ntinued

Region							Number of observations.	Nil.
B.h.	M. open str., 6.	T. retr., 3.	T. rolled, dors. to same cheek, 2.	T. protr. tip to opp. side, 1. M. open, jaw to same side, 1. M. ∠ opp. retract, 1. Lips invert., 1.			15	5
B.i.	M. open str., 7.	T. protr. opp. side, 3.	T. retr., 2.	M. ∠ opp. retr., 2.	M. ∠, same retr., 1. Lips invert., 1.		15	6
B.j.	M. open str., 3.	M. opp. ∠ retr., 2.	M. same ∠ retr., 1. Lips invert., 1.	U.L. opp. elev., 1. Hd. turn, 1.			15	7
B.k.	M. opp. ∠ retr., 1. Lips invert., 1.	U.L. opp. elev., 1.					15	10
C.b.	M. open, jaw to same side, 1.	Mastic., 1.	T. dors. rolled to same cheek, 1.	M. ∠ same side retr., 1.	Pouting, 1.		15	12
C.c.	Mastic., 3.	M. open str., 3.	T. protr., 2.	T. retr., 1.	Pouting, 1.		15	9
C.d.	Mastic., 6.	T. protr. str., 5.	M. open str., 4.	M. open, jaw same side, 2.	S.P. elev. bilat., 1.		15	5
C.e.	M. open str., 7.	T. protr. str., 6.	Mastic., 5.	T. retr., 3.	T. dors. rolled to same cheek, 2. Pouting, 2.	Swallowing, 1.	15	5
C.f.	M. open str., 6.	Mastic., 4.	T. protr. str., 3.	Cheek flattened, 3.	M. open, jaw to same side, 1. T. protr. tip to opp. side, 1. T. dors. to same cheek, 1. U.L. opp. elev., 1.	M. opp. ∠ retr., 1. Swallowing, 1. Puckering, advancing both ∠ mouth, 1.	15	5

T. nued

Square.						Number of observations.	XII.
C.g.	M. open str., 8.	T. dors. to same cheek, 2.	M. open, jaw to same side, 3.	Mastic, 2. / T. protr. str., 2.	T. protr. to opp. side, 1. / M. both ∠ retr., 1. / M. opp. ∠ retr., 1. / Cheek flattened, 1. / U.L. same side elev., 1.	15	3
C.h.	M. open str., 7.	T. dors. to same cheek, 4.	Mastic, 3.	Cheek same side adv., 2	M. open, jaw to same side, 1. / T. protr. to opp. side, 1. / T. retr., 1. / M. both ∠ retr., 1. / M. opp. ∠ retr., 1. / M. opp. elev., 1. / Chin elev. bilat., 1. / Chin opp. side elev., 1. / Pouting, 1.	15	2
C.i.	M. open str., 8.	M. opp. ∠ retr., 3.	M. both ∠ retr., 2.	T. protr. to opp. side, 2.	T. protr. str., 1. / T. dors. to same cheek, 1. / M. both ∠ elev., 1. / M. opp. ∠ elev., 1. / Chin opp. elev., 1. / Mastic, 1.	15	5
C.j.	M. open str., 5.	M. opp. ∠ retr., 5.	M. both ∠ retr., 3.	T. protr. to opp. side, 2.	T. protr. str., 2. / Mastic, 1. / T. dors. to same cheek, 1. / T. retr., 1. / F. inteross. flex, 1. / Th. flexed, 1. / M. same ∠ elev., 1. / M. opp. ∠ elev., 1. / Chin opp. side elev., 1. / Hd. retr., 1.	15	6
C.k.	M. open str., 4.	M. both ∠ retr., 3.	M. opp. ∠ retr., 3.	T. protr. to opp. side, 2. / F. inteross. flex, 2. / Th. flexed, 2.	T. protr. str., 1. / Hd. retr., 1.	15	6
C.l.	Hd. to opp. side, 3.	Sh. add., 1.	M. open, 1.		M. opp. ∠ elev., 1. / Hd. to opp. side, 1. / Sh. add., 1.	15	6

T.   cont ed

Signature	Observations						Number of observations.	Nil.
D.c.	M. open str., 2. / Mastic., 2.	T. protr. str., 1. / M. both ∠ retr., 1.	W. flex., 1. / F. inteross. flex., 1.				15	11
D.d.	Mastic., 4.	M. open str., 3. / T. protr. str., 3.	M. open, jaw to same side, 1. / T. protr. to opp. side, 1.	M. both angles adv., 1. / F. inteross. flex., 1.	Th. flex., 1.		15	9
D.e.	M. open str., 4.	Mastic., 3. / T. protr. str., 3.	T. protr. to opp. side, 1. / T. drawn to same cheek, 1.	T. retr., 1. / M. opp. ∠ elev., 1.	M. both ∠ adv., 1. / F. inteross. flex., 1.	Th. flex., 1.	15	7
D.f.	M. open str., 6.	T. protr. to opp. side, 4. / M. opp. ∠ retr., 4.	Mastic., 3. / T. dors. to same cheek, 3.	M. open, jaw to same side, 1. / M. protr. str., 1.	Swallowing, 1. / M. same ∠ retr., 1.	Pouting, 1. / Both cheeks adv., 1.	15	3
D.g.	M. open str., 10.	M. open, jaw to same side, 4.	T. dors. to same cheek, 3. / Same cheek puckered, 3.	Mastic., 2. / T. protr. to opp. side, 2. / T. protr. str., 2.	M. both ∠ retr., 1. / M. opp. ∠ retr., 1.	M. both ∠ adv., 1. / Opp. chin. elev., 1.	15	1
D.h.	M. open str., 6.	T. dors. to same cheek, 4.	Same cheek puckered, 3.	M. open, jaw to same side, 2. / M. opp. ∠ retr., 2. / M. both angles adv., 2.	Mastic., 1. / M. closes, 1. / T. protr. to opp. side, 1. / Hd. retr., 1.	T. retr., 1. / Opp. chin elev., 1. / Pouting same side, 1. / M. opp. ∠ adv., 1. / M. same ∠ adv., 1.	15	4

TABLE I. continu

Square.							Number of observations.	XII.
D.i.	M. opp. ∠ retr., 5.	M. open str., 2. / T. protr. to opp. side, 2. / M. opp. ∠ elev., 2. / Opp.-chin elev., 2.	Pouting, 2.		M. both ∠ retr., 1. / M. same ∠ elev.. 1. / Lips inverted, I / Hd. retr., 1.		15	4
D.j.	M. opp. ∠ retr., 12.	Ponting opp. side, 3.	T. protr. to opp. side, 2. / M.opp.∠ elev., 2.	Chin opp. side elev., 1. / Hd. to opp. side elev., 1.	M. open str., 1. / M both ∠ retr., 1.	T. retr., 1.	15	1
D.k.	M. opp. ∠ retr., 6.	M. opp. ∠ elev., 4.	T. protr. to opp. side, 2.	M. open. 1. / M. both retr. 1.	Opp. chin elev., 1. / Invert. lips, 1.	M. both ∠ adv.. 1. / Hd. to opp. side, 1.	15	5
D.l.	Hd. to opp. side, 4.	M. opp. ∠ retr., 3.	M. both retr., 1.	M.opp. ∠ elev.,1.	F. ext., 1.		15	7
D.m.	Hd. to opp. side, 4.						15	11
E.c.	M. pouting opp. side, 2.	M. opp. side rolled in, 1.					15	12
E.d.	Mastic., 1. / Elb. flexed, 1.	M. opp. ∠ retr., 1. / Wrist, 1.	M. opp. ∠ elev., 1. / Fingers, 1.	M. opp. ∠ pursed, 1. / Th. ext., 1.	M. opp. side rolled in, 1.	Cheek of same side flattened, 1.	15	9
E.e.	M. opp. side rolled in, 3.	M. opp. ∠ retr., 2. / Th. abd. and flex., 2.	M. both ∠ elev., 1. / M. opp. ∠ elev., 1.	M. both ∠ elev., 1. / M. opp. ∠ elev., 1.	M. opp. side pout, 1. / M. opp. side purs, 1.		15	5
E.f.	Mastic., 2. / T. dors. to same side, 2.	M. both retr., 2. / M. opp. ∠ retr., 2.	M. bilat. pout, 2.	M. open, 1. / M. opp. ∠ elev., 1.	Cheek same side flat, 1. / Th. add., 1.		15	5

TABLE I. continued]

Signals.							Number of observations.	Nil.
E.g.	M. opp. ∠ retr., 7. M. open, 4.	M. opp. ∠ elev., 3. T. tip to opp. side, 3.	Mastic., 2. M. both ∠ retr., 2. M. pursing, 2.	M. open, jaw to same side, 1. T. dors. to same side, 1. M. ∠ same side retr., 1.	Cheek of same side elev., 1. M. bilat. pout, 1. Cheek of same side flat, 1. Th. add., 1.		15	1
E.h.	M. opp. ∠ retr., 3. M. open str., 2.	T. protr. tip to opp. side, 2. M. opp. ∠ elev., 2. Cheek same side flat, 2.	M. open, jaw to same side, 2. Lips same side rolled in, 2.	Mastic., 1. T. rolled over dors. to same side, 1. Chin bilat. elev., 1.	M. bilat. pout, 1. M. bilat. purs., 1. Lips bilat. rolled in, 1.	Cheeks bilat. flat., 1.	15	5
E.i.	M. opp. ∠ retr., 7. M. opp. ∠ elev., 4.	M. open 2. T. protr. tip to opp. side, 2. Lips opp. side rolled in, 2.	T. protr. str., 1. T. dors. rolled to same side, 1.	Opp. chin elev., 1. Elb. flex., 1.			15	2
E.j.	M. opp. ∠ retr., 9. M. opp. ∠ elev., 6.	M. open, jaw to same side, 2. T. protr. tip to opp. side, 2.	Chin opp. elev., 2. Hd. to opp. side, 2.	M. open str., 1. T. dors. rolled to same side, 1. Lips opp. side rolled in, 1.	Elb. flex., 1. W. ext. and pron., 1. Hd. retr., 1.		15	1
E.k.	M. opp. ∠ retr., 8. M. opp. ∠ elev., 5.	T. protr. tip to opp. side, 2. Chin opp. side elev., 2.	Elb. flex., 2. F. flex., 2.	M. open str., 1. T. dors. rolled to same side, 1. Lips opp. side rolled in, 1. Th. flex., 1.	Opp. eyelids closed, 1. W. sup. and pron., 1. Hd. to opp. side, 1.		15	3

TABLE　　　nt　　ed]

Square.							Number of observations.	Nil.
E.l.	Elb. flex., 3.	M. opp. ∠ elev., 2.	M. opp. ∠ retr., 1. Opp. eyelids close, 1.	Eyes to opp. side, 1. F. ext. 1. W. sup., 1. Chin opp. side elev., 1.			15	8
E.m.	Opp. eyelids close, 1.	Sh. rot. in, 1.	Fingers, 1.	Opp. eyelids close, 1. Hd. to opp. side, 1.			15	12
F.d.	M. opp. ∠ elev., 1.	M. opp. side pont., 1. F. ext., 1.	Th. ext., 1.	Th. ext., 1.			15	11
F.e.	M. opp. ∠ retr., 4.	Opp. lower lip depress., 2. Opp. lips roll in, 2. Th. ext., 2.	Mastic., 1. M. bilat. pont., 1.	M. bilat. purs., 1. F. add., 1.	Th. opp., 1. Th. flex., 1.		15	4
F.f.	M. opp. ∠ retr., 4.	M. opp. ∠ elev., 3.	M. bilat. pont., 2. Th. ext., 2.	Th. opp., 1. Elb. flex., 1.	F. ext., 1. W. ext., 1.	Mastic., 1. M. both angles retr., 1. M. bilat. purs., 1. Opp. lower lip depress., 1. Opp. lips roll, in 1.	15	4
F.g.	M. opp. ∠ retr., 6.	M. opp. ∠ elev., 5.	Opp. lower lip depress., 3. Bilat. drawing forward of lips, 3.	M. bilat. purs., 2.	T. protr. tip to opp. side, 1. T. dors. to same side, 1.	Th. ext., 1. F. ext., 1.	15	0
F.h.	M. opp. ∠ retr., 4. M. opp. ∠ elev., 4.	M. bilat. pont., 3.	M. open str., 2. T. dors. to same side, 2.	Mastic., 1. T. protr. tip to opp. side, 1.	Opp. chin elev., 1. Opp. lower lip depress., 1.	Hd. retr., 1. M. bilat. purs., 1.	15	4
F.i.	M. opp. ∠ retr., 7.	Opp. eyelids closed, 4.	Elb. flex., 1.	Hd. retr., 1.	T. protr. tip to opp. side, 1.	T. protr. str., 1.	15	2

TAB. I. continued

Symbols								Number of observations.	Nil.
F.j.	M. opp. ∠ retr., 7.	Opp. eyelids closed, 5.	M. opp. ∠ elev., 4.	Eyelids bilat., blink, 3.	Elb. flex., 2.	Opp. lower lip roll in, 2.	Hd. to opp. side, 1.	15	0
F.k.	M. opp. ∠ retr., 4. Opp. eyelids close, 3.	M. opp. ∠ elev., 4.	Elbow, 2. Hd. to opp. side, 2.	Hd. retr., 1.	Opp. chin, elev., 1. M. purs., L	Opp. lower lip roll.in, 1.		15	6
*	Opp. eyelids close, 3.	M. opp. ∠ elev., 2. Hd. to opp. side, 2.	M. opp. ∠ retr., 1.					15	5
F.l.	Opp. eyelids close, 3.								
F.m.	M. opp. ∠ retr., 2. Hd. to opp. side, 2	Opp. eyelids close, 1. Eyes to opp. side, 1.	F. inteross. flex., 1.					15	12
G.d.	F. opp., 2. Th. opp., 2.	M. opp. ∠ retr., 1. M. opp. ∠ elev., 1.	Opp. lower lip depress., 1.						
G.e.	F. ext., 4. Th. ext., 4.	M. opp. ∠ elev., 2.	M. opp. ∠ retr., 1. Opp. lips roll in, 1.	M. bilat. purs., 1. Opp. lower lip depress., 1.	M. opp. ∠ adv., 1.	W. su W. ex			
G.f.	M. opp. ∠ elev., 6.	M. opp. ∠ retr., 5.	M. ∠ bilat. adv., 2. Th. ext., 2.	M. bilat. pout, 1. M. bilat. purs., 1.	Lower lip de-press., 1. W. flex., 1.	Th. fl			
G.g.	M. opp. ∠ elev., 7. M. opp. ∠ retr., 7.	M. opp. ∠ adv., 3. M. opens, 3.	M. bilat. pout, 2. Opp. lower lip de-press., 2.	T. same side retr., 1. Cheek on same side puckered, 1.	W. ext., 1. Th. ext., 1.	F. ext			

TABLE I. (continued)

Square.							Number of observations.	Nil.
G.h.	M. opp. ∠ elev., 7.	M. opp. ∠ retr., 4.	M. opens, 2. T. protr. tip opp. side, 2.	Th. ext., 2. Opp. cheek puckered, 2.	T. retr., 1. M. same ∠ retr., 1. M. opp. ∠ adv., 1.	M. bilat. pont., 1. M. ∠ bilat. adv., 1. W. ext., 1. F. ext., 1.	15	2
G.i.	M. opp. ∠ elev., 6.	Opp. eyelids close, 4.	M. opp. ∠ retr., 3.	Opp. lips roll in, 2.	W. pron., 1.		15	4
G.j.	Opp. eyelids close, 5.	M. opp. ∠ retr., 4.	M. opp. ∠ elev., 3.	Opp. lower lip roll in, 2.	T. protr. tip opp. side, 1. Eyelids bilat. closed, 1.	F. ext., 1. Th. ext., 1.	15	5
G.k.	M. opp. ∠ retr., 3.	Opp. lips roll in, 2. Hd. to opp. side, 2.	Opp. eyelids close, 1.	Elb. flex., 1. F. ext., 1.			15	8
G.l.	M. ∠ retr., 3.	Eyes to opp. side, 2.	Opp. lower lip roll in, 1.	W. sup., 1.	F. ext., 1.	Hd. to opp. side, 1.	15	11
H.e.	M. opp. ∠ retr., 3.	M. bilat. purs., 1.	Opp. lips roll in, 1.				15	11
H.f.	M. opp. ∠ retr., 6.	M. opp. ∠ elev., 4. Th. flex., 4.	M. bilat. purs., 2. F. flex., 2.	Opp. lips roll in, 1. M. opp. ∠ adv., 1.	Elb. flex., 1. W. sup., 1.	W. ext., 1. Th. add., 1.	15	4
H.g.	M. opp. ∠ retr., 9.	M. opp. ∠ elev., 5.	Th. flex., 3.	Opp. lower lip depress., 2. F. flex., 2.	M. open, 1. M. bilat. purs., 1.	M. opp. ∠ adv., 1. Opp. eyelids closed, 1. Elb. flex., 1. W. sup., 1.	15	3

nued)

						Number of observations.	Nil.
H.h.	M. opp. ∠ retr., 8.	M.opp.∠elev.,6. Th. flex., 6.	Index finger ext., 3.	Opp. eyelids close, 1.	Elb. flex., 1.	15	0
H.j.	Opp. eyelids close, 6.	M. opp.∠retr.,5.	M.opp.∠elev.,3.	Th. flex., 2.	Opp. lips roll in, 1. F. flex., 1.	15	5
H.k.	M. opp.∠retr., 1.	M.opp.∠elev.,1.	Opp. lips roll in, 1.	Opp. eyelids close, 1.		15	12
H.	opp	Opp. ps roll					
H	d. to opp. de.					15	4
I.e.	M. opp. ∠ retr., 4.	Eyes open, 1	Elbow flex., 1.	W. sup., 1.	Fingers, 1. Hd. to opp. side, 1.	15	11
I.f.	M. opp. ∠ retr., 3. M. opp. ∠ elev., 3.	Opp. lower lip depress., 2. Thumb, 2.		Hd. to opp. side, 2.	Opp. lips roll in, l. Fingers, 1. Opp. eyelids close, l.	15	7
I.g.	M. opp. ∠ elev., 6.	Fingers, 5. Thumb, 5.	Opp.eyelids close,4.	M. opp. ∠ retr., 4.	Opp. lower lip de-press, 3.	M. opp. pursed. 1. Hd. retract., 1. Hd. to opp. side. 1.	15	3
I.h.	Thumb, 6.	M. opp. ∠ elev., 4. Fingers, 4.	M. opp. ∠ retr., 3.	Opp. lower lip de-press, 2. Opp. eyelids close, 2.	Hd. to opp. side, 1.	15	6
I.i.	Thumb, 6.	Opp. eyelids close, 3.	M. opp. ∠ retr., 1.	M. opp. ∠ elev., 1.	Opp. lip depress., 1. Elbow, 1.	Opp. lip depress., 1. Sh. l. Fingers, 1. Wrist, 1.	15	8

TABLE I (continued).

Square.					Number of observations.	Nil.
J.f.	Thumb, 3. Wrist, 3. Shoulder, 3. Fingers, 3.	M. opp. ∠ retr., 2.	M. opp. ∠ elev., 1.	Opp. eyelids close, 1.	15	9
			Opp. lower lip depress., 1.	Hd. to opp. side, 1.		
J.g.	M. opp. ∠ retr. 2.	M. opp. ∠ elev., 1. Opp. lower lip depress., 1.	Wrist flex., 1.		15	13
J.h.	M. opp. ∠ retract, 1. M. opp. ∠ elev., 1.	Hd. to opp. side, 1.			15	12
J.i.						
J.j.	Opp. eyelids closed, 1. (Face only observed).					
K.f.						
K.g.						
K.h.						
K.i.					15	14

MARCHES.

Under this heading we wish to describe, in the meaning of the word as originally used by Dr. HUGHLINGS JACKSON, such sequences by movements as we occasionally observed to take place.

At the outset we would state that a great difference characterises the area of representation for the face as contrasted with that for the limbs.   Whereas it was possible in the case of the limbs to make out a definite march for individual parts of the cortex, we have not been able to find sufficient examples of such similarity as to justify us in attributing a definite march to the different parts of the area of representation for the face, but although this exact localisation is not possible the representation of the primary movement, as given in the accompanying list, is such as we have already described in each case.

We have therefore simply appended a list of the sequences of movements or marches arranged in the order in which they most frequently occurred.

TABLE II.

Marches.		Number of squares at which observed.
Primary . . . Secondary . . .	Mouth opens . . . . } Mastication movements, tongue protruded . . . }	7
Primary . . . Secondary . . . Tertiary . . .	Bilateral pouting . . . . } Mouth opens, jaw to same side . . . . . . } Mastication movements . . . . }	5
Primary . . . Secondary . . .	Opposite angle retracted or elevated . . . . } Tongue protruded, tip to opposite side . . . }	5
Primary . . . Secondary . . .	Both angles bilaterally retracted . . . . . } Mouth opens, tongue tip to opposite side . . }	4
Primary . . . Secondary . . .	Mouth retracted to opposite side, opposite lower lip inverted . . . . } Opposite eyelids closed . . . . }	4
Primary . . . Secondary . . .	Mouth opens . . . . } Tongue protruded, tip to opposite side . . . }	3
Primary . . . Secondary . . .	Mouth opens . . . . } Masticatory movements, tongue protruded to op- } posite side, and retracted . . . . }	3
Primary . . . Secondary . . . Tertiary . . .	Pouting or pursing of lips . . . . } Opposite angle retracted . . . . } Tongue rolled to same side . . . . }	3
Primary . . . Secondary . . . Tertiary . . .	Pouting or pursing . . . . } Opposite angle retracted or elevated . . . . } Elevation of opposite lower lip . . . . . . }	3
Primary . . . Secondary . . . Tertiary . . .	Angle of mouth retracted on same side . . . } Mouth opens with retraction of opposite angle . } Mastication with protrusion of tongue . . . . }	2
Primary . . . Secondary . . . Tertiary . . .	Mouth opens straight . . . . } Tongue advanced . . . . } Both angles of mouth retracted . . . . . . }	2
Primary . . . Secondary . . .	Mouth opens wide . . . . } Tongue retracted concave to opposite side, dorsum } to same cheek . . . . }	2
Primary . . . Secondary . . . Tertiary . . .	Opposite lower lip rolled in . . . . } Opposite upper lip rolled in . . . . } Opposite angle of mouth retracted . . . . . }	2
Primary . . . Secondary . . . Tertiary . . .	Pouting and pursing, especially of opposite side . } Opposite lower lip everted and depressed . . } Opposite angle of mouth retracted . . . . . }	2
Primary . . . Secondary . . .	Mouth opens . . . . } Tongue protruded to same side . . . . . . }	1
Primary . . . Secondary . . . Tertiary . . .	Mouth pursed up . . . . } Mouth opened . . . . } Tongue rolled over to same side . . . . . . }	1
Primary . . . Secondary . . .	Pursing of mouth . . . . } Closed mouth drawn to opposite side . . . . }	1
Primary . . . Secondary . . .	Opposite eyelids closed, opposite side of mouth } retracted . . . . } Opposite lower lip inverted . . . . }	1

<center>DESCRIPTION OF PLATES.</center>

NOTE.—In constructing the figures, the actual number of dots represents the precise number of cases in which the movement was elicited at that particular square.

<center>PLATE 8.</center>

Fig. 1. Photograph of the outer surface of the right cerebral hemisphere of the Bonnet Monkey (*Macacus sinicus*). The shaded lines indicate the area which is the object of the present investigation.

<div style="padding-left:3em;">

Sy. = Fissure of SYLVIUS.

R. = Fissure of ROLANDO.

I.P. = Intraparietal sulcus.

Pc. = Præcentral sulcus.

S.F.S. = Superior frontal sulcus.

S.T. = Sulcus frontalis transversus inferior.

Rc.t. = Sulcus retrocentralis transversus.

</div>

Fig. 2. Drawing of the portion of the left cerebral cortex under observation on the paper ruled in squares of 2 millim. side, similar to that used in each experiment. The fissures and sulci indicated are the average of all the twenty experiments, as described in the text. The squares are, as shown, denoted by the double enumeration of capital letters and italics. The lettering for the fissures and sulci are the same as in fig. 1, except *F.t.*, which is the same as *S.t.*

Fig. 3 shows the localisation of the representation of the movement of the closure of the opposite eyelids.

Fig. 4 shows the localisation of the representation of the angle of the mouth of the opposite lip.

Fig. 5 shows the localisation of the depression of the lower lip of the opposite side.

Fig. 6 shows the localisation of the retraction of the angle of the mouth of the opposite side.

Fig. 7 shows the localisation of the retraction of the angle of the mouth of both sides.

Fig. 8 shows the localisation of the flattening of the cheek on the same side.

<center>PLATE 9.</center>

Fig. 9 shows the localisation of the representation of all the movements of the whole tongue. General view. The horizontal lines denote the representation of protrusion of the tongue, with deviation of the tip to the opposite

side; the vertical lines denote that of rolling over of the tongue on its longitudinal axis with the dorsum directed towards the cheek of the same side; the lines sloping forwards and downwards denote that of protrusion of the tongue straight; the lines directed downwards and backwards denote that of retraction of the tongue.

Fig. 10 shows the localisation of the representation of the movement of protrusion of the tongue straight.

Fig. 11 shows the localisation of the protrusion of the tongue with the tip to the opposite side.

Fig. 12 shows the localisation of the rolling over of the tongue with its dorsum to the cheek of the same side.

Fig. 13 shows the localisation of the opening of the mouth straight.

Fig. 14 shows the localisation of the opening of the mouth in which the lower jaw is carried towards the same side.

Fig. 15 shows the localisation of the rhythmical movements of mastication.

Fig. 16 shows the localisation of the elevation of the soft palate.

Fig.3.
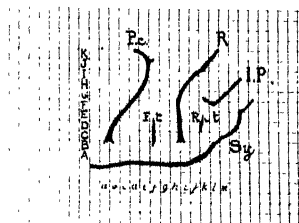
Fig.4.

Fig.5.

Fig.6.

Fig.7.

Fig.8.
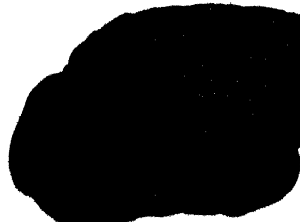
Fig. 11.

Fig. 12.

Fig. 13.

Fig. 14.

Fig. 15.

Fig. 16.

IV. *On some Histological Features and Physiological Properties of the Post-Œsophageal Nerve Cord of the Crustacea.*

By W. B. HARDY, *B.A., Shuttleworth Scholar of Gonville and Caius College, and Junior Demonstrator of Physiology in the University of Cambridge.*

Communicated by Dr. GASKELL, *F.R.S.*

[PLATES 10-13.]

ABOUT two years ago, at the suggestion of Dr. W. H. GASKELL, F.R.S., I undertook the examination of the minute anatomy of the nervous system of the Crustacea. Certain of the results of the investigation are incorporated in the following paper.

The nervous systems of *Branchippus*, of *Astacus*, and of the Zooea larva have been examined, but the following pages are limited, almost exclusively, to the two first-mentioned.

I have to thank Dr. GASKELL and Mr. LANGLEY for the many suggestions with which they have aided the work, and Professor FOSTER, Secretary, Royal Society, for the kindly interest he has shown in my investigations from their commencement, an interest which is extended to all those who have the good fortune to assist him in his professional duties. I am indebted to Mr. BATESON, of St. John's College, for some carefully preserved specimens of *Branchippus* and *Artemia*.

PART I.—THE POST-ŒSOPHAGEAL NERVE CORD OF *Branchippus*.

The central nervous system of *Branchippus* is of special interest as an example of the conditions found in the most primitive Crustacea. It consists of a well-developed supra-œsophageal ganglion, or brain, connected with a small dorsal median simple eye, and a pair of laterally placed compound eyes. This ganglion also innervates the first pair of antennæ. From the brain a pair of longitudinal ganglionated nerve cords pass backwards. These are connected with one another in each segment by a pair of transverse commissures, except in the most posterior part of the animal. The whole nervous system shows many primitive features, it is connected with the ectoderm in many places, shows little trace of fusion in the mid-ventral line, and has nerve cells

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very diffusely scattered about it.   Generally speaking, both the brain and the nerve cords arising from it consist of a central fibrous core, the fibres of which form an exceedingly fine plexus.   This is the "Punktsubstanz" of LEYDIG,[*] and the nerve cells lie wholly on its surface.

Posterior to the œsophagus there are sixteen well-marked ganglia.   These are respectively one mandibular, two maxillary, eleven corresponding to the eleven foliaceous swimming appendages, and lastly, two closely approximated ganglia, situated posterior to the appendage-bearing region, and related to the genital organs. Posterior to these again, in the abdominal region, are two very slightly developed ganglia.   The œsophagus is surrounded by a ring of nervous tissue which forms a single remarkable ganglion connected with the sensory surfaces of the lips and from it a pair of nerves pass to the second pair of antennæ.   There are thus posterior to the brain nineteen ganglia, as follows :—

1. The circumoral ganglion connected with the sensory surfaces round the mouth and the second pair of antennæ.

2. The mandibular ganglion.

3. The ganglion of the first pair of maxillæ.

4. The ganglion of the second pair of maxillæ.

5 to 15. The ganglia of the eleven pairs of swimming appendages.

16, 17. The genital ganglia.

18, 19. The abdominal ganglia.

The ganglia are separated from one another by portions of the cords absolutely unconnected by transverse commissural fibres.

In the *inter-ganglionic regions* each cord is rather rounded in transverse section (fig. 1.), and free from the ectoderm, but as the ganglia are approached a continually closer connection with the ectoderm obtains.   In its free or inter-ganglionic part a delicate sheath with small deeply staining nuclei invests the cord.   Passing from the middle region towards the ganglion above or below, nerve cells become more numerous, always immediately underlying the investing sheath.   One striking fact thus becomes evident in this primitive nervous system, namely, that there is no sharp line of demarcation between ganglionic and inter-ganglionic, or strictly conducting regions.   Further, the fibrous core in this latter region is seen in longitudinal sections to consist of longitudinally arranged fibres which stain well with carmine stains. Connecting these, however, in many cases are more delicate filaments which run obliquely, so that the whole instead of being merely a bundle of parallel fibres, may be more exactly described as a plexus of which the longitudinal strands have been relatively exaggerated.

The shallow groove on the ventral surface of the animal and between the nerve cords deepens considerably just posterior to the mouth, and is there lined by elongated sense cells, and abundantly supplied with nerve filaments, thus forming a sensory pit

* LEYDIG, 'Handbuch d. vergleich. Anatomie,' vol. 1, Tübingen, 1864.

between the mandibles. In the thoracic region the groove is very shallow in the inter-ganglionic regions, but deepens between the ganglionic enlargements. In this groove is a line of sense cells on either side of the mid-ventral line. They are especially prominent near the ganglia. We may, therefore, say that there exists on the ventral surface a pair of almost continuous sense lines, situated on the ridge bounding the ventral groove, and perfectly segmented in correspondence with the ganglionic segmentation of the cords. They meet just posterior to the mouth in a deep sense pit or groove (fig. 2), which again, in its turn, may be regarded as a backward continuation or thickening of the sense ring round the mouth. The arrangement of these ventral sense cells will be seen to have an important bearing on the disposition of the sensory elements of the cord. In the inter-ganglionic, as well as in the ganglionic regions, filaments pass from these ventral sense cells to the cells of the cord.

Structure of a Typical Ganglion.

For purposes of description I will choose the ganglion related to the third pair of swimming appendages. It consists of a double thickening of each nerve cord, so that on either side are anterior and posterior swellings, each connected with its fellow of the opposite side by a transverse commissure. Seen in transverse section (figs. 4, 5, and 6), each of these thickenings is oval, the long axis being horizontal, and from the outer border of each a distinct nerve passes. Each ganglionic swelling, besides being intimately connected with the subjacent ectoderm, abuts dorsally against the gut.* Between the anterior and posterior swellings on each side, and lying partly wedged in between the lateral extension of the oval nerve cords and the ectoderm, are a pair of large vacuolated cells with large granular deeply-staining nuclei (fig. 6). These are the peculiar segmental excretory glands described and figured by Professor CLAUS.† The anterior and posterior portions of the ganglion present profound differences in the arrangement of their elements, and these differences are constant throughout the entire thoracic region.

The Anterior Half of the Ganglion (figs. 4 and 5).

At its anterior end the ganglion commences by an increase in the number of the cells on the internal edge of the cord; these rapidly expand into a well-marked group of multipolar cells. The delicate membrane previously described as enveloping the cord becomes discontinuous over these cells, allowing them to extend themselves both underneath the cord, and also beyond the cord laterally, always in most intimate

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* Similarly, the pre-œsophageal ganglion or brain lies beneath and in close contact with a pair of anteriorly directed diverticula of the mesenteron.

† CLAUS, "Untersuch. über d. Organisation u. Entwickel. v. *Branchippus* u. *Artemia*." 'Arb. aus Wien,' vol. 6, p. 267.

connection with the ectoderm. There is thus formed a *plate of cells* which extends under the entire ganglion, dying out at either end only when the inter-ganglionic cord loses its close and immediate connection with the ectoderm. From the most external of these cells delicate processes pass, which branch and end in various ectoderm cells on the ventral surface external to the ganglia, and furnished with sense hairs, and we have here every intermediate condition between ectoderm cells which are almost continuous with nerve cells, to ectoderm cells connected with the ganglion cells by comparatively long processes (fig. 4). From their connections with the ectoderm this ventral group of nerve cells would appear to be sensory in character, and to form a sensory system which does not give origin to a definite sensory nerve. They are connected with the imperfectly segmented series of sense cells which occur on the ventral surface in close connection with the nerve cords.

On the inner dorsal angle of each of the anterior ganglionic swellings is a cell group (figs. 4 and 5, int. dorsal cells), the cells of which give off processes to the anterior commissure, and are also connected by a dorsal series of arcuate fibres with a third group of cells on the outer dorsal edge of the anterior ganglionic swelling (*ibid.*, ext. dorsal cells). These external dorsal cells, though abutting on the external cells of the ventral sense plate, yet differ from them in size and in the fact that from them a bundle of fibres passes through the ganglionic plexus to form the anterior part of the anterior commissure (fig. 4). The posterior part of the anterior commissure is composed of fibres which traverse the ganglionic plexus and pass directly out as part of the out-going nerve bundle (fig. 5). These decussating fibres thus divide the plexus of each anterior swelling into dorsal and ventral portions, of which the latter is finer and denser in character.

We can therefore distinguish in each half of the anterior portion of the ganglion three cell groups: (1) the ventral group, which extends under the entire ganglion; (2) the external dorsal group, from which decussating fibres pass, forming the anterior portion of the anterior commissure; (3) the internal dorsal group. The external and internal dorsal groups are connected by a bundle of dorsal arcuate fibres.

The *anterior pair of nerves* plunge immediately into the lateral mass of muscles, and, turning dorsalwards and outwards, pass into the upper part of the appendage, giving off motor fibres to the various muscles as they pass them. These end immediately in the neighbourhood of one of the nuclei of the curious granular protoplasmic sheath which, more or less completely, envelopes the transversely striated contractile portion of the muscle fibre (fig. 7). The anterior pair of nerves thus are mainly, if not entirely, motor. Careful searching with a $\frac{1}{12}$th objective failed to discover a case of undoubted connection with the ectoderm. Fibres can be traced into each anterior nerve from both the external and internal dorsal cell groups of its own side. These arise either directly from the cells, or as lateral branches from processes of the cells which are lost in the plexus. A bundle of fibres also passes directly from the

posterior portion of the anterior commissure directly across the plexus into each of the anterior nerves.

We have already seen that the fibres of the anterior commissure not only contribute to the formation of the anterior nerves, but also pass to the two dorsal cell groups. It is thus possible that the directly decussating fibres of the anterior nerves are in part connected with the dorsal cell groups of the opposite side.

Some of the fibres of each anterior nerve may be traced directly into the more dorsal portion of the ganglionic plexus. These probably are connected with nerve cells by lateral branches, but the connection cannot be seen in sections. It is probable from the fact that a large number of the fibres of the anterior commissure end in the plexus, that a crossed connection exists between each anterior nerve and the plexus of the opposite anterior ganglionic enlargement.

The following, then, are the central connections of each anterior nerve :—

1. With both external and median dorsal cell groups of each side, but mainly with those of the same side.

2. With the dorsal plexus of each side.

Finally it should be remembered that the dorsal cell groups of the same side are connected by the dorsal arcuate fibres.

These dorsal cells and the dorsal coarser fibrous reticulum I regard as the motor portion of the anterior half of the ganglion.

It may be well here to emphasize the fact that the elements of the entire ganglion are placed in the most intimate connection by means of the complex central plexus. No conception of this primitive nervous system will be an adequate one which does not realize the fact that it is throughout a cell and fibre plexus condensed along certain lines. Even the well-marked bundle of fibres passing from the commissure direct to the anterior nerve is, in its course through the ganglionic plexus, intimately related to, and obviously a part of, that structure, being connected with it by filaments.

The *posterior half of the ganglion* (fig. 6) is very different in its arrangement from the anterior part just described. It consists, like the former, of a swelling on each nerve cord, which is oval in transverse section, and of a pair of nerves, one on each side, arising from their lateral aspects. The posterior ganglionic enlargements, like the anterior swellings, are connected by a transverse commissure. But while the anterior commissure lies in close contact with, and is intimately related to the ventral ectoderm (fig. 4), the posterior commissure arches freely above it.

The cells of the posterior ganglionic swelling on each side form three groups :—

1. A median group placed at the junction of the commissure with the cord (fig. 6, median group). Each cell gives off a strongly marked process to the commissure, while, on the external side, it contributes processes to the ganglionic plexus. The commissure stretches between these cells, and appears to communicate only with

them, neither contributing directly to the formation of the posterior nerves, nor sending fibres directly into the plexus, as is the case with the anterior commissure.

2. An external lateral group of cells which give off marked cell processes into the plexus, while on their outer side they give origin to part of the outgoing nerve.

3. The ventral cells before mentioned which are continuous with the ventral cells found beneath the anterior portion of the ganglion and have the same connections.

The *posterior nerves* pass outwards, but take a course much more ventral than that of the anterior nerves. They pass below the muscles and immediately over the large excretory cells. Each is composed of two bundles (fig. 6) which are respectively dorsal and ventral. The ventral and larger ramus curves downwards and is distributed to the ectoderm of the ventral lobe of the appendage ; the dorsal ramus passes outwards and upwards to be distributed to the more peripheral ectoderm. The fibres of the ventral ramus arise from the lateral cell group. The dorsal ramus arises directly from the ganglionic plexus. Within the plexus the processes from the median (commissural) cell group passing outwards, and those from the nerve direct, and from the external cell group radiating inwards, almost meet (fig. 6).

Those fibres of the nerve which pass to the more peripheral ectoderm, that is, the fibres forming the dorsal ramus of the posterior nerves, do not go direct to their destination, but enter, at any rate in some cases, multipolar cells, the processes from which radiate to the ectoderm cells. Such a cell is shown in fig. 8. This relation is extremely interesting and suggestive when taken in connection with the known relations of sporadic nerve cells in Vertebrates, where such cells are regarded as being distributing or multiplying centres for centrifugal impulses. In this case, however, the contrary occurs, for these cells in *Branchippus* act as *condensing centres for centripetal impulses.* Similar "condensing" cells form a distinct layer in the optic ganglion of *Branchippus*, one process passing from each cell to the brain while several enter the cell from the eyes. Yet another consideration is suggested by these condensing sensory cells, which are mostly suspended in the body cavity by their processes. The dorsal ramus of the posterior nerve, which is characterised by the possession of such cells, passes to parts more removed from the central nervous system than does the ventral ramus. At the same time, its fibres, instead of being given off from cells, arise directly from the ganglionic plexus. Thus we may regard the ganglion cells from which the fibres of the ventral ramus take origin, as having travelled inwards from positions more remote from the nerve cords, and this process has led to their becoming an integral part of the central nervous system. Such a view has this advantage, that it accords with the many features of the primitive system of *Artemia*, which point to a derivation from a much more diffuse cell and fibre plexus by a process of condensation.

Each of the posterior nerves is therefore composed of two parts (1) a dorsal bundle, which springs directly from the central ganglionic plexus, and has ganglion cells on

tho course of its fibres; and (2) a ventral bundle, which arises from the lateral group of nerve cells.

There is yet another group of nerve cells which, like the ventral sensory cells, cannot be said to belong specially to either the anterior or posterior divisions of the ganglion. In describing the anterior part of the ganglion I mentioned a group of cells, the internal dorsal cells, situated on the median dorsal surface and connected with the anterior commissure on the one hand, and with the external dorsal cell group on the other, and contributing directly to the formation of the motor nerves. With this internal dorsal cell group a column of cells is connected (fig. 6), at any rate in point of position. They extend backwards over the region between the anterior and posterior ganglionic thickenings, and over the latter, to finally die away posterior to the ganglion. From this group of cells scattered fibres pass which are lost amid the body muscles. The latter are situated internal to the muscles of the appendages, and form masses closely applied to the sides of the gut (fig. 6). I often suspected the connection of some of these fibres with the gut itself, and am inclined to believe that a few, at any rate, form the nerve supply of the mesenteron. However that may be, they mainly constitute the motor supply of the body as opposed to the appendage muscles, and form a motor system comparable in its antero-posterior extension to the sensory system connected with the ventral lines of sense cells. I stated above that the ventral sensory system owed its antero-posterior extension to the fact that it is related to longitudinal lines of sense cells. Similarly, this dorsal diffuse motor system appears to me to owe its antero-posterior extension to the fact that it is connected with a group of muscles, the flexors and extensors of the body, which extend from end to end in almost unbroken series, and are not clearly divided into disconnected myomeres related to the successive body segments.

Summary.

1. The central nervous system of *Branchippus*, taken as a whole, consists of two cords of nervous tissue running the length of the body, and connected anterior to the mouth by the brain, in the region of the mouth by the circumoral ganglion, and posterior to the mouth by the transverse commissures which connect the various ganglionic enlargements.

2. Each nerve cord is comprised within a delicate nucleated sheath, and consists of a mass of fine nerve fibres invested more or less completely by nerve cells. The investment of nerve cells is most complete in the brain, and more or less wanting in the inter-ganglionic regions of the cords.

3. The ganglionic regions differ from the inter-ganglionic regions chiefly in the development of a very fine plexus on the ventral aspect of the cords. The dorsal portion of the cords in each ganglion is mainly a direct continuation of the inter-ganglionic fibres, and is, therefore, largely conducting in character. The ganglionic regions are also characterised by the large number of nerve cells.

4. The inter-ganglionic and ganglionic regions are not sharply distinct from one another. Nerve cells extend from the latter to a considerable distance along the former, and a plexiform arrangement of the fibres is not wholly wanting in the inter-ganglionic regions.

5. The nerve cells have the following connections :—

(a) They send their processes wholly into the plexus. These cells occur mainly in the brain and pre-œsophageal cords. They give off one or several processes into the central plexus. In fig. 3 we have a section through a cord in the pre-œsophageal region, and it will be seen that the cells have a rounded external surface, and give off numerous processes into the central plexus. Where the cell layer thickens, however, those most removed from the plexus fuse, as it were, all their processes into one, and thereby become unipolar cells with one thick process which ultimately breaks up in the plexus.

(b) They give off an axis-cylinder filament to a peripheral nerve on the one side, while on the other side they are connected with the central plexus, either by branching filaments, or by a single process which breaks up into that structure. Such cells appear to lie on the course of afferent fibres. Cells belonging to this group are, in many cases, pyramidal in shape, and give off from the base of the pyramid several processes to the skin, while from the apex of the pyramid only one process passes to the central plexus. Such cells act as "condensing centres" for centripetal impulses.

(c) They give off a peripheral axis-cylinder process to end in a muscle, while from the principal process, or from the cell itself other finer processes arise which end in the central plexus. Such cells are disposed dorsally in the central nervous system, and are connected with the outflow of efferent fibres.

(d) Pyramidal cells giving off on the one side a single fibre to the posterior commissure, while from the other side filaments pass into the central plexus.

6. The distribution of the elements in a typical ganglion is as follows :—

(a) On the ventral surface of the ganglion is a group of sensory ganglion cells, the fibres from which are connected with a line of sense cells on the ridges bounding the mid-ventral groove, and which form an almost continuous linear series exaggerated beneath each ganglion. These two lines of sense cells are a backward extension of the folded sense ring which encloses the mouth, and they meet just posterior to the mouth, where the otherwise shallow ventral groove narrows and deepens to form a sense pit.

(b) On the internal dorsal angle of the cords is a group of cells, from which are derived the motor fibres to the body muscles. These, like cell group (a), extend over both the anterior and posterior portions of the ganglion, and for some distance along the inter-ganglionic cords.

(c) On the internal and external dorsal angles of the *anterior* half of the ganglion are groups which are connected with the fibres of the anterior pair of nerves appendage muscles.

(d) On the internal angle of the posterior half of the ganglion is a group of cells

which give origin to the fibres of the short ventral ramus of the posterior nerves. Similar cells lie scattered on the course of the fibres of the longer dorsal ramus. These cells are connected with afferent fibres.

(*c*) On the internal angle of the *posterior* half of the ganglion is a group of cells from which the fibres of the posterior commissure arise.

The anterior and posterior halves of the ganglion present the following striking structural differences :—

1. The anterior commissure is closely connected with the skin. The posterior commissure arches freely above the skin.

2. The anterior commissure does not lie stretched between two cell groups. Its fibres mainly plunge directly into the "Punkt" substance, and many of them continue as a well-defined bundle across that structure to pass out in the anterior nerves. The posterior commissure lies stretched between two cell groups (group *e.*)

3. A large number of the fibres of the anterior commissure continue as a well-defined bundle across the central plexus, to form part of the anterior nerves. Or, in other words, a considerable number of the fibres of the anterior nerves directly decussate. The fibres of the posterior nerve do not decussate.

The identification which has been made above of certain elements as the afferent and efferent mechanisms of the nervous system of *Branchippus* finds further support if we turn to the structure of the circumoral ganglion, or ganglion of the stomodæum and second pair of antennæ. A complete description of this ganglion would lengthen this communication unduly, and, moreover, would be mainly of morphological interest. The facts which lead to the identification of the motor and sensory elements may, however, be briefly set down.

The circumoral ganglion consists of a double ring of nervous matter enclosing the œsophagus and uniting the nerve cords. The double ring is displaced so that it lies at a considerable angle with the horizontal plane of the body, this displacement being due to the very great hypertrophy of the upper lip and the continuation of the œsophagus as a horizontal and posteriorly directed tube which ends posteriorly in the mouth. The double ring is especially developed posterior to the œsophagus, where it lies stretched as a double commissure between a ganglionic enlargement on each nerve cord. In this, the commissural portion of the ring, the outer part runs across from nerve cord to nerve cord, as a bundle of regularly arranged parallel fibres, resembling the commissures of the typical ganglion. The fibres of the commissural portion of the inner ring also run for the most part regularly, but still a plexiform arrangement is very obvious. This inner ring is very closely connected with the sensory surfaces of the mouth, and it also shades off into a plexus of filaments connected with the epithelium lining the œsophagus. In point of fact, it may be very accurately described as a local hypertrophy or condensation of this œsophageal sensory nerve plexus. The outer ring, on the other hand, is connected

with the innervation of the well-developed muscles of this region, and may similarly be described as a local development of a nerve-plexus connected with the musculature of the œsophagus. Having thus established the fact that the inner ring is sensory and the outer motor, can we proceed a step further and identify the two rings with the two parts, anterior and posterior, of the typical ganglion? The connections of the commissural portions, that is, those thicker portions which lie on the posterior surface of the œsophagus stretched between the ganglionic enlargements on each nerve cord, enable us to do this with certainty. The anterior and posterior commissures of the typical ganglion present, as we have seen, certain well-defined structural characters. The anterior commissure, unlike the posterior commissure, does not lie stretched between two cell groups. On the contrary, its fibres plunge directly into the ganglionic plexus. The commissural portion of the external ring of the circumoral ganglion presents this feature. On the other hand, the posterior commissure of the typical ganglion does lie stretched between two cell groups, and a similar connection characterizes the commissural portion of the inner ring of the circumoral ganglion. Thus the outer motor ring of the circumoral ganglion presents the same structural features as the anterior portion of the typical ganglion, and a similar structural agreement is found between the inner sensory ring and the posterior portion.

If we turn to the nerves arising from the circumoral ganglion further confirmatory evidence is obtained. The circumoral ganglion gives origin to the nerves of the second pair of appendages, the second antennæ. Two nerves pass to each second antenna, and they arise from the cords a short distance anterior to the ganglion. CLAUS,[*] in his account of the anatomy of *Branchippus stagnalis*, describes these nerves, and traces the anterior nerve on each side to the muscles of the second antennæ, and the posterior nerve to the sense cells of those organs, and I am able to confirm him in this respect. Tracing these two nerves centrally we find that the motor pair are connected with the motor, or external, commissure, in a way precisely similar to the direct connection which we have seen to exist between the anterior commissure and anterior nerves of the typical ganglion. Similarly there is evidence that the fibres of the posterior or sensory nerves to the second antennæ are connected with those portions of the ganglionic enlargements of each cord which correspond in structure to the posterior swellings of the typical ganglion, and which are placed in connection by the inner sensory commissure.

PART II.—THE POST-ŒSOPHAGEAL NERVE CORD OF *Astacus fluviatilis*.

The abdominal region of the central nervous system of *Astacus* contains six ganglia, related respectively to the six metameres into which the abdomen is divided. The

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[*] 'Zur Kenntniss d. Baues u. d. Entwickel. von *Branchippus stagnalis* u. *Apus cancriformis*,' Göttingen

first five of these ganglia differ from one another only in certain small details, and a description of one will serve as a description of the whole.

The *Second Abdominal Ganglion* forms a marked enlargement on the cord, the bulging being most prominent ventrally. There arise from it two pairs of nerves which I propose to distinguish respectively as the anterior and posterior nerve pairs. From the external dorsal aspect of the cord just posterior to the ganglion, a nerve arises on each side, thus forming a third pair to be distinguished as the posterior dorsal nerves.

Distribution and Character of the Nerves.—The *anterior pair* pass directly outwards beneath the great flexor muscles of the abdomen (fig. 9). Their point of origin is not only more ventral than that of the other nerves, but they also pursue a more ventral course. They run outwards almost at right angles to the longitudinal axis of the body, in the groove formed by that thickened portion of the sternal carapace which extends transversely under the ganglion, and between the bases of the second pair of abdominal appendages. Immediately anterior to these nerves is a vertical slip of muscle passing down from the great flexors of the abdomen to be inserted into the thickened carapace (fig. 9*a*). Very shortly after leaving the ganglion each nerve divides into two branches, which, however, continue in the same sheath for some distance. They separate from each other just before the appendage is reached (fig. 9). The posterior and smaller then turns downwards into the appendage, the anterior and slightly larger divides into three main branches on the anterior border of the base of the appendage. These again divide into smaller branches which are distributed to the appendage muscles, pleuron, and dorsal skin. The posterior branch is distributed to the comparatively small muscles which lie within the terminal part of the appendage, but by far the greater number of its fibres pass to the skin which, in the males, is extremely sensitive, the first and second pairs of abdominal appendages being modified to form copulatory organs. On their course the anterior nerves give off a few fine branches to the sternal skin.

Fibres.—The anterior nerves are composed of two markedly different classes of fibres (figs. 11 and 18),

  (1.) Large bold fibres with nucleated sheaths, and
  (2.) Fine filaments.

The large fibres may be again divided into (*a*) large fibres with thin sheaths, (*b*) small fibres with thick sheaths. In a section through the nerve (fig. 11), it will be seen that these different classes of fibres are segregated into distinct bundles. Each of the large fibres is bounded by a distinct nucleated sheath, which, as was pointed out by KRIEGER,* encloses a substance of such extreme fluidity, that under the influence of preserving agents its shrinks into small clots here and there in the course of the fibre. There is no trace of a sheath resembling the medullary sheath

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* KRIEGER, "Centralnervensyst. d. Flusskrebses," 'Zeits. f. wiss. Zool.,' vol. 33.

of Vertebrate nerve fibres, either in preparations treated with osmic acid, or in those stained by WEIGERT's method.

In tracing the anterior nerves into the ganglion a curious point is noticed, namely, that the transverse sectional area of the whole nerve on each side is much reduced, thus forming a narrow neck just before the nerve plunges into the ganglion. If the fresh nerve is examined under the microscope, or if individual fibres are carefully followed in a series of sections through this part, the sudden increase in the bulk of the nerve which takes place a fraction of a millimetre from the ganglion, is found, as has already been shown by HAECKEL,* to be due to the fact that the large fibres there divide into large and medium-sized fibres (fig. 12). In the example figured it will be seen that while the smaller branch is conspicuously smaller than the nerve fibre before the branching, the large branch is of much the same size.

Both branches of each anterior nerve contain fibres of each of the two classes, as will be seen on reference to fig. 11.

The *posterior ventral nerves* arise directly from the ganglion posterior to, and slightly above the origin of the anterior nerves. Instead, however, of passing outwards at right angles with the cord, and at a level anterior to the appendages, they trend outwards and backwards (figs. 9 and 10), running on the ventral surface of the great flexor muscles to the region immediately posterior to the attachment of the appendages. Their position in this part of their course is not so ventral as that of the anterior nerves. They lie at a level between the slips of the flexor muscles which pass to the sternum in each segment and the main mass of those organs. Each nerve, shortly after leaving the ganglion, divides into two branches which run in the same sheath for some distance. The anterior and smaller branch separates from the main trunk of the nerve where the latter curves abruptly round the belly of the flexor muscles to pass dorsalwards. It then turns upwards and outwards, to run along the infolding of the carapace, which forms the joint between abdominal segments 2 and 3, and is lost in the extensor muscles (figs. 10, 10a, 10b). The main trunk of the nerve passes sharply backwards along the outer surface of the belly of the flexor muscles and appears on the dorsal surface of those muscles about the middle of the *third* abdominal segment. It gives off the following branches :—

(a) To a thin sheet of the external extensor muscles which spreads out into the pleuron of the third segment, and to the dorsal skin in that region.

(b) Branches which pass above and end in the external extensor muscles, but also give off twigs to the dorsal skin.

(c) Branches which pass beneath the external extensors to end in the coiled

muscles consist of four distinct bands situated on each side of the

* Beiträge zur Flusskrebses," 'Müller's Archiv,' 1857, Taf. XVIII., figs. 2

mid-dorsal line. The two median bands, or internal extensors, as I have called them, are coiled on themselves so as to form roughly a spiral of muscle ; the external extensors, on the contrary, are flat band-like muscles with slips passing off to be inserted into the tergum in each body segment (fig. 29).

The posterior ventral nerves of the second abdominal ganglion, therefore, are distributed to the extensor system of muscles in the posterior two-thirds of the third abdominal segment, and the anterior third of the fourth segment. Professor CLAUS* describes a similar distribution of the nerves to the body muscles in the abdominal region of Nebalia.

The different branches when they reach the extensor muscles divide continuously, forming horizontal fan-like tufts of fibres, thus forming a plexus which spreads through a considerable length of the muscles.

The posterior ventral nerves are composed of two sizes of fibres like the anterior pair, but the small fibres form only a small part of each nerve (fig. 14).

The *dorsal nerves* are of extraordinary interest. They spring from the dorso-lateral portions of the cord just posterior to the ganglion (fig. 16), and turning backwards are distributed to the great flexor muscles (fig. 26).

Each of the dorsal nerves near its origin, and before any branching has taken place, is composed of only a few, generally ten, very large tubular fibres, about 30 to 60 μ in diameter.

In order to adequately realize the size of these fibres, it should be remembered that they have only a thin sheath. The dimensions given above should therefore be compared with those of the axis cylinders of vertebrate medullated fibres rather than with the whole fibre. Each fibre is distributed to a relatively enormous mass of muscle by a process of continuous branching. No small fibres occur in the main nerve, but there is usually a small bundle composed wholly of a few of the small thick sheathed class of tubular fibres (fig. 15) which leaves the main trunk immediately after its exit from the cord and curves abruptly ventralwards. I have not followed it further.

The fibres when they enter the cord form ascending and descending columns, seven of the ten fibres passing upwards to the second abdominal ganglion, and three passing downwards to the third ganglion. In other words, the dorsal nerves of the second ganglion, though belonging mainly to that ganglion, yet derive part of their elements from the ganglion next below.

Like the posterior ventral nerves the dorsal nerves branch very extensively and irregularly, and pass to a part of the flexor muscles which lies mainly in the third abdominal segment.

We thus see that the body muscles, both flexors and extensors, have been displaced posteriorly, the relation of the second abdominal ganglion to the second pair of abdominal appendages affording us a fixed point for reference. And this displacement has taken place to such an extent that the main branch of the posterior ventral

* CLAUS, "Ueber d. Organismus der Nebaliden," 'Arbeit. Wien,' vol. 8, 1889.

nerves of the second abdominal ganglion courses on the dorsal surface at the junction of the *third and fourth segments* on its way to the extensor muscles (figs. 10A and 10B).

The same posterior displacement of the muscles is shown when we examine the nerves of the remaining abdominal ganglia. In connection with the general question of metamerism, it is interesting to notice that the very definite blood supply of these muscles does not appear to have suffered a similar displacement. The dorsal aorta gives off at about the middle of each segment two pairs of arteries, one on each side to the extensor muscles, and one passing ventrally on each side of the flexor muscles.[*]

Motor and sensory fibres.—Before turning to the ganglion itself it will be advisable to discuss the question of the identification of motor and sensory fibres in the nerves.

Evidence derived from the distribution of the different classes of fibres.—The chief tactile organs possessed by *Astacus* are long hair-like filaments, each composed of a special expansion of the general cuticle jointed to the carapace, and containing a filamentous process of an ectoderm cell in its axis. These lie mainly on the appendages, but also occur on the edges of the pleura, the telson, and the posterior edge of the tergum in each segment. The general surface elsewhere is covered by a thick and, for the most part, hard lamellated horny cuticle, which in sections is seen to be traversed by comparatively infrequent delicate pores in which fine processes of certain elongated ectoderm cells lie. These are especially well seen in sections through the skin near the anus (fig. 17). The tergal surface, where the carapace is especially thick, is relatively insensitive, while the swimmerets, on the contrary, with their numerous tactile hairs, are especially sensitive. In correspondence with this we find that the nerves supplying the swimmerets contain a very large number of fine fibres, while the nerves to the tergal surface contain relatively fewer; and that the branch of the anterior nerve which passes *into* the appendage and to the small muscles therein contained contains relatively fewer large fibres than does the larger branch which innervates the larger muscles of the appendage (fig. 11). Further, the nerves passing to the telson, which contains no muscular elements, and to the sensitive region round the anus, are entirely composed of fine fibres. On the other hand, the nerves passing solely to the flexor muscles, that is, the posterior dorsal pair in each segment, contain only the large tubular type of fibres.

Evidence derived from direct experiment.—Seeing that the anterior nerves of the abdominal ganglia contain a considerably larger number of the fine nerves than do the posterior ventral nerves, I determined to try the relative effects of stimulation.

The abdominal cord was laid bare from the ventral surface, the operation requiring great care to avoid injuring the very delicate and superficial anterior nerves. The posterior nerves were then stimulated with an interrupted, or tetanizing current, and the following points noticed:—

(1.) The anterior nerve of the left side was stimulated at a point about midway between the ganglion and the appendage. The effects were double, (*a*) flexure of the limb, and, (*b*) enormous reflex disturbance of the animal generally, every appendage being moved. In other words, the main effect of stimulating this nerve was to produce very great sensory disturbances.

(2.) The posterior ventral nerve on the same side was stimulated with the same strength of current, and the only effect was a faint sensory disturbance and feeble movement of the second abdominal appendage of the same side.

The difference between the central effects produced by stimulating these nerves is very obvious when the animal is exhausted. Then, when stimulation of the anterior nerve on one side will produce reflex movements of all the appendages, stimulation of the posterior nerves leads to no such result.

The movement of the appendage which resulted from stimulation of the posterior nerve I regarded as being due to reflex action, and to settle this point the following experiment was made :—

(3.) The posterior ventral nerve was cut and its peripheral end stimulated. No movement of the appendage resulted, but, on applying the electrodes to the central end, results occurred similar to those described under (2).

(4.) With respect to the posterior ventral nerves, MARSHALL* states that on stimulating the peripheral end of one of them (in the Lobster) no effect was produced. Hence he concluded that "the anterior nerve would seem to be mixed, but the posterior nerve purely sensory." This result was due to the fact that, in his experiments, the animal was fixed in such a position that it was impossible to observe any contraction of the extensor muscles. If, however, the animal be placed in such a position that the extensor muscles can be observed while the posterior nerve is being stimulated near the ganglion, and therefore on the ventral aspect of the body, a marked tetanus of the extensor muscles is found to follow stimulation.

(5.). The posterior dorsal nerves in the case of *Astacus* are so delicate and deep lying, and their unbranched free portion is so extremely short, that it is impossible to directly stimulate them without escape of the current into the muscles. The experiment was therefore carried out in the following manner :—The anterior and posterior ventral nerves were cut on both sides and in each segment of the abdomen. Thus only the posterior dorsal nerves were left intact. The nerve cord was then cut in the thoracic region and lifted on to the electrodes. On opening the circuit the abdomen was sharply flexed.

The facts both of dissection and stimulation thus lead to the conclusion that the anterior nerves are of mixed function, and contain both afferent and efferent fibres. But they mainly supply sensory surfaces. The posterior ventral nerves are also of mixed function, but they supply a relatively larger mass of muscles, and a much more

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* MARSHALL, "Some Investigations on the Physiology of the Nervous System of the Lobster."
' Studies from Owens College, Manchester,' 1886.

insensitive region of skin. In correspondence with this difference between these two pairs of nerves, we find that the anterior nerves are relatively poor in the large tubular fibres, but contain a very great number of the fine fibres. The posterior ventral nerves, on the other hand, are relatively much richer in large tubular fibres.

The posterior dorsal pair differ from the anterior and posterior ventral nerves, in being solely efferent in function, and we find that they contain only the large tubular fibres.

We may thus, I think, conclude that the large tubular nerve fibres of *Astacus* are efferent, while the fine nerve fibres are afferent in function ; and these two classes of fibres are not only sharply marked off from one another in point of size, but also the gulf between them is unbridged by intermediate forms within the limits of the somatic system.

Summary.—Three pairs of nerves arise from each of the first five abdominal ganglia :—

(1.) An anterior pair, which arise directly from the ganglion, and contain a large number of the fine, or afferent fibres, and comparatively few of the large, or efferent fibres. These supply the appendages with motor and sensory fibres, and also the skin of the sternum and pleura.

(2.) The posterior ventral nerves containing relatively more large fibres. These supply the dorsally placed extensor muscles and the dorsal skin in the third segment, or segment next following.

(3.) Posterior dorsal nerves which are purely motor and innervate the flexor muscles.

The General Relations and Structure of the Second Abdominal Ganglion.

Only a very general mention of the main features of the ganglion itself need be made, since KRIEGER has so fully dealt with them in his paper on the "Central-nervensystem des Flusskrebses" ('Zeit. f. Wiss. Zool.,' vol. 33, p. 527). The ganglia appear as "small knot-like swellings on an apparently single longitudinal commissure." Each ganglion is surrounded by a tough lamellated membrane, which is separated from the nerve substance by a loose reticulum, especially abundant on the ventral face of the ganglion. The spaces in this connective tissue reticulum are large and are filled with blood. We thus have two distinct sheaths, a point not sufficiently insisted on by KRIEGER (fig. 18), of which the inner one is practically a system of blood spaces bridged by connective tissue, and is only slightly developed in the inter-ganglionic regions (fig. 23).

Owing to the continuity of the investing membrane, the nerve cord at first sight appears to be single, but the nervous elements in reality form two perfectly distinct cords, which are united only in each ganglion by a thick transverse bridge. In the inter-ganglionic regions the two halves are separated by a median vertical lamella of

connective tissue. As they enter a ganglion either above or below they diverge from one another, and the inner sheath at the same time becomes thicker. There is thus formed at each end of the ganglion a vertical cleft-like space between the lateral masses of nervous tissue, and filled by the inner sheath of the cord; and the anterior cleft, or *anterior fissure*, is separated from the *posterior fissure* by the transverse bridge of nervous tissue which was spoken of above.

The outer sheath.—This sheath is composed of one or more lamellæ, each of which is built up of fine fibrils which appear to be bound into bundles (fig. 19). These may either run parallel to one another, or interlace. The lamellæ appear to be entirely free from cellular elements in their substance, but on the surface of the sheath flattened plate-like cells occur (figs. 19 and 20), with large, flattened, deeply-staining nuclei.

Quite on the inner face of this sheath these cells are more numerous and their plate-like extensions often overlap one another (fig. 19). Supporting filaments are seen to arise from the outer sheath which are continuous with the inner sheath (fig. 20).

The inner sheath appears in sections as a coarse reticulum, largely cellular in nature. It closely resembles in structure the tissue surrounding the sternal artery, and lying beneath and about the nerve cord. The inner sheath is essentially a blood-containing investment, and into its spaces the arteries supplying the ganglion open. In the inter-ganglionic regions of the cord this sheath is very thin. The branched cells of the inner sheath are continuous, on the one hand, with the flattened cells on the inner face of the outer sheath; and on the other, with the variously modified cells which form the intimate supporting structure of the nervous elements.

The *supporting tissue of the nervous structures* appears to be mostly, if not entirely, cellular in character, and is best seen by teasing out the fibres of the inter-ganglionic commissures where they diverge into bundles on entering the ganglion. If these have been preserved with FLEMMING's fluid mixed with an equal volume of ·5 per cent. solution of osmic acid, and afterwards stained with hæmatoxylin, the individual bundles will be seen to be covered by an imperfect sheath of flattened cells with large, oval, deeply-staining nuclei (fig. 21). Cells of this type represent the imperfect attempts at endothelium building found so widely in *Astacus*. They occur, only of much larger individual size, lining certain of the large body spaces, and similar cells have been already noticed in connection with the outer sheath of the ganglion. Within the substance of the ganglion, where the nerve fibres branch and become much finer, similar supporting cells occur. Each consists of a small cell body, from which arise short plate-like processes which branch into the wildest tangle of exceedingly delicate filaments (fig. 22), thus recalling the neuroglia cells of the Mammalia. There is thus formed a supporting tissue composed, like the neuroglia of Vertebrates, of the delicate processes of much modified cells. Both plates and filaments are of an optically structureless and non-staining character, very different

from the finely granular and staining cell substance of the nerve cells. Their nuclei also stain more deeply than the nerve cell nuclei, and are not so coarsely granular.

. *Blood supply.*—The ganglion is supplied with blood by four arteries which arise by a short common stem, which springs from the posterior sternal artery, immediately under the middle of the ganglion. After penetrating the external sheath, two of the arteries curve round the sides of the ganglion between the roots of the anterior and posterior nerves, and, running in the inner sheath, finally end in the dorsal portion of an enlargement of that sheath on the lateral face of the nervous tissue, and above and between the two nerve roots. These may be called the *lateral arteries.* The other two arteries run respectively anteriorly and posteriorly for a short distance, and then make their way upwards through the anterior and posterior fissures to the dorsal surface, where they open into a dorsal median thickening of the inner sheath. They may be called the *anterior and posterior arteries.*

Thus, in the intact ganglion, there must be a stream of blood flowing through the interstices of the inner sheath from the dorsal to the ventral side, where it drains into a large ventral sinus situated in the inner sheath, and incompletely divided into four longitudinal sinuses by septa. The blood finally leaves the ganglion by apertures placed in the mid-ventral line at the posterior end of the ganglion, and leading from the ventral sinus of the ganglion into the ventral abdominal sinus. The arrangements for the nutrition of the large nerve cells which occupy the ventral, and, to a less extent, the lateral surfaces of the nervous tissue of the ganglion, are most interesting. These cells are of the unipolar, pear-shaped type, and are quite removed from the dense nerve substance of the ganglion. Each is covered by a delicate cellular sheath, and this alone separates the cell substance from the blood ; for they may be said to hang in bundles suspended by their processes, and steadied by the reticulum of the inner sheath, in blood spaces of that tissue. Between them, in the mid-ventral line, and below them, in the posterior region of the ganglion, are the venous sinuses mentioned above. ·

The mass of the ganglion is composed of a fibrous reticulum, coarse in some places, fine in others, and the fibres in the finer reticulum appear to touch one another, so that blood spaces are conspicuous by their absence. In the case of nerves and of commissures the same fact strikes one, whether they are viewed in the fresh condition, or examined by means of sections—the sheathings of the nerve fibres are contiguous with one another, and, at first sight, no provision appears to have been made for their nutrition. This may, I think, explain the prevalence of "tubular fibres" in the central nervous system and peripheral nerves of *Astacus.* Each of these fibres in section appears as a tubular nucleated sheath, and little more. The contents of the tube, or what corresponds to the axis cylinder of the nerve fibres of Vertebrates, have shrunk into small clots gathered here and there at long intervals on the course of the fibre. In other words, the contents of such a tubular fibre are exceedingly fluid. If they are examined in the fresh state bubbles of air may often be seen, which may

be made to move about in the almost fluid contents, as though one were dealing with a fine tube filled with fluid. If these fibres are isolated and watched, their contents will, as was pointed out by KRIEGER,* be seen to undergo a change comparable with *rigor mortis.* Clotting takes place, the clot appearing in the form of granules, which outline delicate fibrils, which I regard with KRIEGER as the true axis-cylinder fibrils. These, I take it, are suspended in life in an extremely fluid substance, protoplasm or plasma, by the aid of which the transportation of nutritive material or the removal of waste matters can be managed through considerable lengths of these tubular fibres. It is therefore possible that each efferent fibre is the morphological equivalent of a considerable number of afferent fibres, each one of the latter being, without doubt, a single axis cylinder.

Be this as it may, it is, at any rate, abundantly clear that the disposition of the nerve cells on the surface of the dense nervous tissue of the ganglion, and their rela-tion to the blood streams, lends no support to the idea that they are nourished, even in part, by their processes. The distinction of the processes of nerve cells into "nutritive processes" and true nerve or axis-cylinder processes has been advocated by NANSEN† for the Crustacea, and by GOLGI for Vertebrates. In the latter case the distinction is based upon histological facts which appear to me to be adequately explained by the effect of the shrinkage of the tissue in occluding the lymph lacunæ which, we must suppose, surround the nerve cells of the central nervous system and their processes during life.

Arrangement of the Nervous Elements of the Ganglion.

These consist of cells, fibres, and fibrillar plexus. The relation of the cells and fibrous elements is the same as that which is found in the nerve cord of *Branchippus* where the former lie wholly on the surface of the latter. In the cord of *Astacus,* however, cleft-like spaces filled with the inner or blood-containing sheath penetrate the nerve substance, and the cells to a certain extent occupy these (fig. 34, *c*). The nervous tissue of the ganglion is composed of fibres and fibrillar plexus and nerve cells. The fibres are of the same tubular character as those already noticed in the nerves. They run in the ganglion for the most part in longitudinal bundles, which, penetrating the ganglionic plexus, divide it up into regions. Many of these fibres, especially in certain of the bundles, run straight through the ganglion without effecting connection with its elements. The other tubular fibres in the ganglion are either commissural between the lateral halves, or derived from the large unipolar cells which occur in the ventral cell-plate mentioned above.

In passing up the cord towards the brain one finds, as in Vertebrates, that the number of fibres passing straight through the ganglia to regions below continuously increases. Such fibres are limited to the more dorsal portion of the cord, and in the

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* *Loc. cit.*
† NANSEN, 'Bergen's Mus. Aarsberet,' 1886.

upper portion of the thoracic cord they may be readily separated by simple dissection from the ventral bundles of fibres which pass to and through the ganglionic substance. This is well seen in fig. 24, which is a drawing of the ganglion of the chelæ, as seen from the ventral surface.

The ganglionic plexus is separated into regions distinguished both by the size of the fibres forming the plexus and the complexity of their arrangement. We may distinguish two grades :—

    (a) A coarser plexus, the elements of which, though much smaller than the tubular fibres of the inter-ganglionic cord or nerves, yet show the marked tubular appearance (fig. 36).

    (b) A fine plexus, comparable in the extreme tenuity of its elements to that of the nerve-cord of *Branchippus* (fig. 32). The fine plexus and the coarse plexus are, respectively, ventral and dorsal, and related, the former to the fine nerve fibres, and the latter to the large nerve fibres. The fine plexus may be said generally to form an irregular plate on the under surface of the fibrous portion of the ganglion.

The large tubular nerve fibres pass at once to the coarse plexus, and we may regard the latter structure as being formed by their branching. I do not, however, regard the coarse plexus as constituting the final ending of the large nerve fibres, but merely as the place where those fibres subdivide before passing to various regions of the more ventral fine plexus. The fine nerve fibres, on the contrary, run in bundles directly to the fine plexus (figs. 32 and 34). The fine plexus, as will be seen later, is not a homogeneous structure, but presents differences in density in its different parts.

The Internal Connections of the Anterior Pair of Nerves.

Each of the anterior nerves, as it enters the ganglion, divides into a dorsal and a ventral root, each of which contains both large and small fibres (fig. 35). In each root the large and small fibres form quite distinct bundles, and in the section through the trunk of this nerve, figured in fig. 13, it will be seen that a short distance from the ganglion the fine and large fibres are still arranged in distinct bundles.

The *dorsal root* passes to a distinct mass of plexus which lies in the outer, or more lateral, part of its own half of the ganglion in the anterior region, at the level of the entry of the anterior nerve, and immediately above the ventral root (fig. 33, d, c). This mass of plexus is very definitely divided into a small rounded and ventral mass of fine plexus, and a dorsal mass of coarse plexus, which extends to the lateral surface of the ganglion above the entering nerve. (Fig. 33, d, c. The dotted line bifurcates, and ends respectively in the coarse and in the fine plexus.) The fine fibres of the dorsal root pass to the ventral fine plexus, the large fibres to the dorsal coarse plexus.

I regard this root as being of mixed function, motor and sensory, and its centre as being composed of two parts : (1) a dorsal coarser portion, formed by the first branching

of the large efferent fibres; and (2) a ventral, very much finer portion, to which the fine afferent fibres pass directly.

Connection with Cells.—By far the greater number of the fibres, both large and small, pass directly to break up in the fibrous reticulum. Where the large fibres arch round the ventral ball of "punkt" substance on their way to their own coarser reticulum, I found several distinct T-shaped junctions. One limb of the T could be traced to the most external of the ventral unipolar cells in the region of the entering nerve. These form a distinct group of cells of fairly uniform size, and averaging from 30 to 40 μ in the shortest axis.

In addition to these, there are cells situated between the bundles of entering nerve fibres, where they diverge. These are much smaller. Each gives off one process which proceeds to strike the entering bundles at right angles. I think I may say positively that nerve fibres do not end in these cells, though their processes are connected by T-shaped junctions with certain of those fibres—probably the fine ones (fig. 35, *a*, *a'*).

In longitudinal vertical sections it is seen that these cells lie chiefly in two masses anterior and posterior to the entering nerve, and that their fine processes pass in two main bundles to join the fine fibres before they break up into the "punkt" substance (fig. 35, *a*, *a'*). There is a third very distinct group of cells related to the dorsal root, and lying on the dorsal side of the entering nerve, and on the lateral surface of the coarse nerve network (fig. 36). Each cell receives an axis-cylinder process, and gives off from its internal face processes into the plexus of the ganglion. The small tubular fibres, with thick neurilemma, pass to these cells. This cell group is continuous with the more dorsal cells of the lateral extension of the ventral plate of cells between the point of exit of the anterior and posterior ventral nerve, and, from them, fine tubular processes pass, which curve round as dorsal arcuate fibres (fig. 36).

The *ventral root* also consists of large tubular and fine fibres. It passes in beneath the centre for the dorsal root, and the fine fibres break up in a large mass of fine plexus in the most ventral part of the ganglion near the middle line (fig. 33, *v.c.*). The large fibres pass up anterior to the fine fibres to break up in a mass of coarse plexus dorsal and anterior to the above-mentioned fine plexus (the region it will occupy is indicated by the upper limb of the dotted line in the figure). This root, therefore, also contains motor and sensory elements connected respectively with a coarse and fine plexus.

Cells.—The large fibres appear to be connected by short branches given off at right angles, with a group of the ventral unipolar cells immediately underlying them, just after their entrance into the ganglion (fig. 35, *b*). The fine fibres are connected with the group of small nerve cells lying between the divergent roots (fig. 35, *a*, *a'*).

To sum up the internal connections of the anterior pair of nerves, we see that each nerve divides into two parts on entering the ganglion. Each part, or root, is composed of large tubular and fine fibres which pass respectively to a coarse and fine

ganglionic plexus. There are thus two distinct centres related to the two roots. These centres are connected with one another by zones of plexus, and by unbranched fibres in such a way that the coarse plexus of the external centre (that of the dorsal root) is connected with the coarse plexus of the more median centres, and, similarly, the fine plexus of the external centre is connected with the fine plexus of the median centre. Further, the centres of the two sides of the ganglion are placed in communication with each other by two commissures, one of which consists of the finest fibrous elements, and stretches between the median masses of fine plexus. The other consists of coarser elements and connects the median coarse plexus of the one side with that of the other. The completely dual nature of the internal connections of the anterior pair of nerves, that is, the nerves to the appendages, I take to be of especial significance. In the thoracic region, where the nerves to the appendages are large and predominate, the dual nature of their internal connections is even more strikingly shown. In the thoracic region, also, the two roots of the appendage nerves, instead of being fused in the neighbourhood of the ganglion, and only diverging within its sheath, form two long roots which remain quite distinct as far as the second joint, *i.e.*, a considerable distance into each appendage (fig. 25).

These two roots differ markedly in size, and, as a result of experiments carried out on the Lobster, MARSHALL (*loc. cit.*) states that (1) " the small nerve contains motor fibres which supply the extensor muscles of the limb, and especially the divaricator muscle of the claw ;" and (2) "the large nerve contains the motor fibres to the muscles which raise the limb and close the claw." Also the small nerve contains "afferent fibres which cause reflex contraction of the claw through the large nerve which supplies the occlusor muscle," while the large nerve contains " afferent fibres which cause opening of the claw by reflex action through the small nerve which alone supplies the divaricator muscle." These experimental results I have been able to substantiate in the case of the nerves to the chelæ of *Astacus*. I have further found that the small nerve passes to a median centre, the large nerve to an external centre in its ganglion.

In contradistinction to the posterior ventral and dorsal nerves the fibres of the anterior nerves do not appear to directly decussate in any case. RETZIUS failed to find any decussating fibres ;* but, as has been pointed out, bilateral connection is made by fibres large or small between the various centres.

Concerning the cell connections of the fibres of the anterior nerves we find that :—

(1.) The large tubular efferent fibres are connected with large nerve cells.

(2.) The fine fibres, afferent in function, are connected with small nerve cells. The connection with the cells is, as RETZIUS (*loc. cit.*) has already shown, by lateral branches. The nerve cells, therefore, do not lie in the direct path of the nervous impulses to or from the ganglionic plexus. Exceptions to this appear, however, to occur in the case of the small tubular fibres with thick neurilemma,

* RETZIUS, 'Biologische Untersuchungen,' Stockholm, 1890.

Internal Connections of the Posterior Pair of Nerves.

The first and most striking facts in connection with the posterior nerves is that they do not pass to two distinct centres, and that a certain number of the fibres, as RETZIUS also found, do directly decussate.

On entering the ganglion the fine fibres of each nerve take up a position posterior to the large fibres, and form a well-defined single stream which passes to a single mass of fine plexus on the ventral surface and towards the median line (fig. 34, *f.pl.*). This centre, therefore, occupies the same relative position as the internal centre, or centre for the ventral root, of the anterior nerve. Sometimes the fine fibres enter the nerve tissue of the ganglion at a point higher up than the large fibres, and arch round to pass to the ventral-lying fine plexus. They may be said generally to take a more or less arched course through the nerve substance of the ganglion and to enter their centre from above. Certain of these fine fibres directly decussate and pass to the centre of the opposite side. This, however, does not always occur.

The large fibres form two sets, some ascend, and after passing for a short distance as external arcuate fibres, plunge into the nerve substance of the ganglion and either—

(*a*) Break up in a zone of coarse plexus lying at the junction of the upper and middle third of the ganglion; or

(*b*) They directly decussate, passing to a similar region on the opposite side (fig. 34, *a*).

The second stream of large fibres (fig. 34, *b'*) passes in more ventrally and anteriorly, and communicates with a cell group lying on the ventral surface of the ganglion and extending round the entrance of the posterior nerve (figs. 34 and 35, *b*). Some of the fibres, however, break up directly in a mass of plexus, lying anterior and external to the fine plexus related to the fine fibres of the posterior nerves.

We thus see that the fine fibres or sensory elements of each posterior nerve pass to a single centre, while the large fibres or motor elements are distributed to two regions of coarse plexus. I take it that we may correlate this dual character of the central connections of the motor elements with the fact that the nerve supplies two sets of muscles—the external and internal extensors, differing enormously in their general arrangement, and in the position of their fibres. At the same time these two masses of plexus are intimately connected by unbranched fibres and bridges of plexus.

RETZIUS (*loc. cit.*) has shown that a certain number of the fibres of the posterior nerves have no connection with the elements of the ganglion, but turn directly backwards to descend in the longitudinal commissure. A similar arrangement, as will be seen, obtains in the case of the posterior dorsal nerves. On the other hand, fibres have not been traced directly from the commissures to the anterior nerves.

Internal Connections of the Posterior Dorsal Nerves.

These, as will be remembered, arise from the dorsal and external surface of the inter-ganglionic cord, a variable but short distance posterior to the ganglion. As was pointed out before, they each consist of two parts derived respectively from the ganglion above and the ganglion below. This fact is only demonstrable by sections, since simple dissection merely reveals the fact that they arise a very short distance posterior to, but in very close connection with the second abdominal ganglion. I have examined these nerves in detail in the first five abdominal ganglia. Each nerve is composed of from ten to thirteen tubular fibres, which vary in size from 12 to 13 μ.

On entering the nerve-cord, or a little distance before entering it, each nerve divides into two unequally sized roots, of which the smaller, containing generally three fibres, passes down the inter-ganglionic commissure to the ganglion next below. It at first lies on the external dorsal angle of the cord, but soon makes its way obliquely over the external giant fibre* to a more median position, and then curves ventrally to run in the external region of the dorsal group of longitudinal fibres. These I propose to call the *descending root*. The remaining fibres, eight to ten in number, turn sharply upwards and form a column of fibres external to the external giant fibre. These form the *ascending root* (fig. 23).

Tracing the whole bundle in its upward course to the second ganglion, it is found to assume a more dorsal position until it overlies the external giant fibre. Almost before any indications of ganglionic structure have appeared in the cord, the most median and largest fibre (a, 35 μ) detaches itself from its fellows and passes obliquely forwards and inwards immediately under the internal giant fibre. It continues its course across the middle line to the opposite half of the ganglion, where it divides into two main branches. One is given off ventrally immediately after the decussation has been completed. It runs downwards and backwards, at first in the dorsal sulcus between the lateral halves of the cord in this region, and then plunging into the nerve substance ends in or rather forms the single process of one of the largest giant cells, situated in the external and anterior part of the posterior division of the ventral plate of nerve cells (fig. 33, a). It will thus be seen that the most internal fibres of each side, after decussating, each give off a branch which runs forwards for a considerable distance before passing to its cell.

The rest of the fibre, or the other branch, continues horizontally across, under the internal giant fibre of the opposite side, to finally break up in a small mass of dorsally situated plexus which overlies the nerve substance between the two giant fibres. This plexus is intimately related to a small group of small nerve cells on its dorsal surface.

Continuing anteriorly, a second fibre (b, 18 μ) next detaches itself from the bundle

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* In each lateral half of the inter-ganglionic cord are two large giant fibres which are respectively external dorsal and internal dorsal. They lie completely dorsal to all the other fibres.

and runs inwards under the internal giant fibre, then curves downwards and decussates by the dorsal motor commissure, which is largely composed of the decussating motor fibres of the posterior pair of nerves (fig. 34, c). It breaks up in the dorsal coarse plexus of the opposite side.

Two fibres, one 18 μ (c) the other 15 μ (d), are the next to leave. They take different courses. The first arches close round the external giant fibre and runs inwards a short distance towards the median line. It then turns downwards and forwards, running in a sulcus or fissure between two columns of longitudinal fibres which I will call the internal dorsal and median dorsal columns (cf. later). It then appears to divide into two branches, of which one turns inwards towards the median line, and breaks up in the dorsal plexus of its own side, in the region corresponding to that of the opposite side in which fibre (b) is lost.

Fibre (d) leaves the bundle at the same time that fibre (c) does and curves *outwards* over the external giant fibre, then passes forwards and downwards on the external surface of the nerve tissue, and breaks up in the most lateral portion of the dorsal plexus of its own side, and at a level lying between the points of origin of the anterior and posterior nerves. It is the only fibre which passes laterally instead of mesially, and from its superficial and isolated course can be easily traced.

Two fibres (e, 12 μ, and f, 12 μ) leave next and turn inwards and downwards, to curve under the external giant fibre and pass to the same lateral-dorsal region as fibre (d).

The three last-mentioned fibres (d), (e), and (f) thus take the same course.

Two fibres (g, 20 μ, and h, 20 μ) alone remain, and they turn abruptly downwards and outwards to curve down ventrally between the entering anterior and posterior nerves, and end in two large unipolar cells of the ventral cell-plate on the same side corresponding in position to the cell of the opposite side in which fibre (a) ended. Each gives off a branch to the dorso-lateral plexus as it passes near it. They thus completely resemble fibre (a) in their connections, except in the fact that they do not decussate.

The following table summarizes the connections of the descending column of the posterior dorsal nerve on each side.

It will be seen that the fibres fall in three groups defined by distribution and size.

Group 1.—Three large fibres which break up in the coarse plexus on most dorsal aspect of the ganglion.

$$\text{Fibre } a \text{ decussates} \quad . \quad . \quad . \quad 35\,\mu.$$
$$\left.\begin{array}{c} \text{"} \quad g \\ \text{"} \quad h \end{array}\right\} \text{do not decussate} \quad . \left\{\begin{array}{l} 20\,\mu. \\ 20\,\mu. \end{array}\right.$$

Group 2.—Three fibres, of which one, the largest, arches above and others below the external giant fibre to pass to the lateral portion of the dorsal plexus.

Fibre d passes above external giant fibre   15 μ.

" e } pass below external giant fibre . { 12 μ.
" f }   { 12 μ.

Group 3.—Two fibres which break up in the more lateral portion of the dorsal plexus.

Fibre b decussates . . . . .   18 μ.
" c does not decussate   .   18 μ.

The table shows that there is a close agreement in size between fibres having the same connections.

The Motor System of Nerves.

When we consider together the central origin and mode of distribution of the motor fibres, we see that they present certain features of remarkable interest. Their relations are most clearly shown, because the peculiarities are most exaggerated in the case of the motor system of the flexor muscles just described.

The unit of the system is a large unipolar cell, characterised by the abundance and solidity of its cell substance, which is loaded with granules of a basophile nature. Like basophile granules generally, they colour more or less intensely with osmic vapour. The cell is enclosed in a nucleated sheath, and suspended by its process in the blood stream of the ganglion.

The single process from this cell runs for a considerable distance without giving off branches. The first branches leave the main process in the ganglion, and break up in the general plexus of that structure. These relations are very clearly shown in the figures illustrating RETZIUS's work (*loc. cit.*). The cell process then leaves the nerve cord in one or other of the nerves and, on its course to the muscle, it branches *into a great number of fibres which pass to a large mass of muscle fibres.* In the case of the nerves to the very large flexor muscles each myomere (fig. 26) may be innervated by as few as ten nerve fibres, which arise from the same number of nerve cells.

The fibres branch dichotomously, but one of the branches is smaller than the other, while the larger branch is equal in size to the fibre before the branching. This is shown at fig. 12, and, as it occurs in the more peripheral parts of the system, in HAECKEL's beautiful drawings (*loc. cit.*). By this method of branching *the transverse sectional area of the unit of the motor system continuously increases as one passes from the nerve cell to the final ending in the muscles.**

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* These connections have been traced by RETZIUS (*loc. cit.*) with the aid of methylene blue; and by myself in sections and dissections.

The whole structure, which we may call the unit of the motor system, is enclosed in a continuous nucleated sheath which forms the capsule of the nerve cell, and the "tube" of the tubular process.

The substance of the processes is very different from the substance of the cell. The latter is solid and granular, the former is extremely transparent (compare HAECKEL, *loc. cit.*, figs. 6, 8, 10, 11, 12), and consists of two parts :—

(1.) Fine filaments running longitudinally; and suspended in

(2.) A more or less fluid plasm (KRIEGER, *loc. cit.*). The junction between cell substance and process substance is extremely abrupt. Similar tubular fibres enclosing filaments are described by SCHIEFFERDECKER and KOSSEL in *Petromyzon*,* and the facts at present at our disposal warrant the suggestion that the filaments are the structures which convey the nervous impulses.

The unit of the motor system of *Astacus* thus consists of the following parts :—

(1.) A single nerve cell which, from its histological characters, and relation to the blood stream, appears to be a highly metabolic structure ; and which is removed by a considerable length of nerve from the direct track of the nervous impulses.

(2.) A single nerve process from this cell which branches in a characteristic fashion, and consists of a number of filaments, presumably processes of the cell, which are suspended in a plasma.

(3.) The branches of this process, and, therefore, of the single nerve cell. These are very numerous, are distributed to the plexus of the ganglion and to a very large mass of muscle fibres.

If the prevailing conception of the trophic functions of the central nervous system is correct, we must regard this single nerve cell as the trophic centre for this large mass of muscle fibres.

Though at first sight the motor system of the flexor muscles, consisting, as it does, in each metamere of only a few fibres and cells, seems a simple one, yet a consideration of the arrangement of the flexors themselves shows that we must regard it as a simple contrivance arranged so as to secure a complex result. Figs. 26, 27, and 28 represent dissections of the flexor muscles, and it is there seen that each myomere extends over three metameres. The contraction of the muscles produces flexure of the abdomen, and, if we define the terms "origin" and "insertion" to mean respectively the fixed points from which the muscle pulls and the point of attachment which is moved by the contraction, then we may say that each myomere has three origins and one insertion. Taking the myomere, the main mass of which lies in the fourth abdominal segment, for description, its most anterior origin is from the transverse thickening of the sternum in the second abdominal segment, and it is inserted into the anterior edge of the transverse thickening of the sternum in the fifth abdominal segment (figs. 9, 26, and 28). From the anterior origin to the insertion the muscle runs as a mass of tissue, with a great thickening in the fourth abdominal segment, and with an S-shaped curl, arranged

* SCHIEFFERDECKER u. KOSSEL, 'Gewebelehre d. Menschlichen Körpers,' figs. 129 and 130.

in such a way that the fibres in the thickest part of the muscle run almost transversely to the long axis of the animal (figs. 10A, 10B, 26, and 28). The other two origins are situated in the fourth abdominal segment and serve to steady the muscle. They are (*a*) a tendinous attachment to the similar muscle of the opposite side by means of a ventral sheet of fibres springing from the most superficial aspects of the belly of the myomere (fig. 28), and (*b*) an origin from the pleuron of the same side by a dorsal sheet of fibres (figs. 26, 27, and 28). The ventral sheets of fibres are best developed in connection with the myomeres of the second and fifth abdominal segments.

It is thus clear that we are dealing with a muscular machine of very great complexity, and one the proper working of which must depend upon the coordination of the contraction waves in different regions of the large mass of tissue in respect to the time when they occur.

The mode of innervation of the electric apparatus of the Torpedo helps us to form a first mental conception of how this correlation is accomplished. In the electric organ the correlation is the simplest possible, namely, the discharge of the individual batteries at the same instant. This, as WAGNER has pointed out, is accomplished by the agreement in the length which intervenes between the first branching of the fibre and the final end of each filament. In the case of the flexor muscles of *Astacus* the correlation is a threefold one—

(1.) A sequence in the time of contraction of the large and distinct masses of fibres which in sections are seen to compose each myomere;

(2.) The contraction at the same instant of time of the fibres of any one mass, and

(3.) The serial contraction of the separate myomeres from before backwards.

In case (2) the simultaneous contraction of a large number of fibres receives the simplest explanation, if we suppose that they are innervated by one unit of the motor system, and in this connection I would again draw attention to the constant relation which obtains between the size of the fibres and their morphological relations, and that the distance along the nerve fibre from the central origin of the impulse to where it breaks on the individual muscle fibres is the same. In other words, the explanation would exactly resemble that put forward by WAGNER to account for the simultaneous discharge of the batteries of the electric organ. The sequence in the time of contraction of the various fibre masses of the myomere would receive the simplest explanation if we referred it to *differences* in the length of fibre interposed, in the case of each unit of the motor system, between the central origin of the impulses and the muscle fibres.

Lastly, the sequence in the contraction of the different myomeres we might regard as a phenomenon of central origin and to be referred to the time occupied in the transmission of the disturbance from the higher centres down the abdominal nerve cord.

It will be remembered that in describing the motor arrangements of a typical

ganglion of *Branchippus* allusion was made to a diffuse motor system connected, not with the purely metameric appendage muscles, but with the longitudinal body muscles which simply send slips to the skin in each segment.   And it was there pointed out that this diffuse system consisted of a column of cells on the median dorsal aspect of the cord, from which fibres passed to these muscles.   One cannot fail to see in the motor system of the abdominal body muscles of *Astacus* a distinct reproduction of these conditions in *Branchippus,* for the nerves, both to the extensor and flexor muscles of the abdomen, that is, to the body muscles, each arise from two ganglia.

The Inter-ganglionic Regions, or Longitudinal Commissures of the Cord.

The longitudinal commissural parts of the cord are composed solely of tubular fibres running parallel to one another and of very various sizes.   As has been already noticed, the two lateral halves in the inter-ganglionic regions are clearly marked off from one another by a median vertical septum of connective tissue.   There is absolutely no admixture of what may be called ganglionic elements, that is, of nerve cells or plexus, with the commissural fibres ; on the contrary, the inter-ganglionic regions correspond, accurately and solely, to the white matter of the spinal cord of Vertebrates (fig. 23).   As has been mentioned previously, many of the fibres continue straight through the ganglion to regions below.   In the abdominal region, and in sections passing through those parts of the inter-ganglionic cords more removed from the ganglia, a grouping of the fibres into columns is not very obvious, owing to the slight development of the inner sheath.   On approaching the ganglia, however, whether from below or from above, a distinct division into columns is very apparent.   The fibres in different regions diverge from one another, while at the same time the median fissure widens, and the inner sheath becomes thicker, in order to fill the spaces thus formed, which appear in transverse sections as fissures, or sulci, occupied by an inward extension of the inner sheath (fig. 18).   It should be distinctly stated that there is no confusion of the columns of fibres along the whole inter-ganglionic tract ; there is no decussation or branching of fibres into this column or that, but, on the contrary, they maintain a parallel course without branching, the different columns simply diverging from one another at the upper or lower limits of the ganglia.

The result of this divergence is that the transverse sectional area of the cord rapidly increases as the ganglion is approached.   This increase, however, is also due to another cause, namely, that in each column some of the fibres branch.   In one case the fibres were counted in one lateral half of the cord about a quarter of the distance between the first and second abdominal ganglia away from the latter ; and again, in a section taken through the region where the divergence of the columns passing to the second ganglion had taken place.

The number of fibres of all sizes found in the hemisection furthest from the ganglion was 613, in the hemisection near the ganglion 815 ; while in the former case the

large fibres numbered 161, and in the latter 225. The division of the fibres may be readily observed in longitudinal sections, or teased preparations, and it is then seen to take place *wholly without the intervention of cells.* Passing on into the ganglion the division of certain of the fibres in each bundle is continued until they form tufts of fine fibres, which, just before they enter the ganglionic tissue, form a plexus, the fibres of which lie mainly in the general direction of the bundle. The transverse or obliquely running filaments also are rather finer, so that, at any rate near where it merges into the parallel bundles of fibres of the inter-ganglionic cord, it may be described as a *nerve plexus, the longitudinal fibres of which are the more prominent.*

In describing the inter-ganglionic region of the ventral chain of *Branchippus* in the first part of this paper, I said that it consisted of a fibrous core of longitudinally arranged fibres with oblique filaments, and a study of the primitive nervous system strongly impressed me with the idea that it is derived from a nerve plexus by the condensation of that plexus along certain lines and in certain places. The connections between the nerve fibres and plexuses described above I regard as suggesting this phylogenetic origin in the case of the nervous system of *Astacus.* In the Zooea of a Crab (one of the Paguridæ) which I have examined, the ganglia in the thoracic region are practically continuous. There is no inter-ganglionic cord, but a continuous internal plexus. Here and there in this are delicate strands composed of only a few fibres, and traceable right up to the brain. These represent the great longitudinal columns of the Mammalian cord, and appear to end in the thick sheath of nerve cells surrounding the fibrous core. The longitudinal commissural columns or fibres between ganglion and ganglion have not yet appeared. In other words, the general plexus still performs the functions carried out in the Mammalian cord by the root zones of white matter, and probably, in part, by the grey matter itself, and in *Astacus* by the parallel fibres of the inter-ganglionic regions.

As the growth of the Zooea proceeds, the ganglionic masses, the cells and fibrous elements of which are at first quite continuous, are, as it were, pulled apart from one another, to remain connected by an inter-ganglionic zone of parallel fibres.

We may, with RETZIUS, distinguish three connections for the longitudinal fibres of the ventral cord :—

(1.) Directly with the plexus of the ganglion.

(2.) Directly with the plexus of the ganglion, but with a T-shaped junction, with cells.

(3.) With cells whose processes merge in the plexus of the ganglion (fig. 30). The connection through cells is more common in the higher parts of the cord.

Paths of Conduction in the Cord.—In transverse sections through the cord just above or below the ganglion, the fibres in each half of the cord are clearly seen to lie in three main columns—dorsal, median, and ventral. Each of these again is divisible into three regions—internal, median, and external (fig. 18). The ventral columns are related chiefly to the centres of the anterior nerve, the median to the centres of the

posterior ventral nerves, and the dorsal to the dorsal nerves. The divisions of the respective columns which abut on the median fissure contain a larger proportion of fibres, which run through the ganglia without change. Thus the great conducting columns, that is, those fibres which correspond in the cord of *Astacus* to the fibres of the pyramidal tracts, and GOLL's column in the cord of Vertebrates, form, in the abdominal region, a mass of fibres, wedge-shaped in transverse section, and disposed symmetrically on each side of the median fissure. The base of the wedge is dorsal. Higher up the cord, in the thoracic region, these fibres occupy a still more dorsal position (fig. 24). Further, these fibres are stratified according to their connections, those related to the appendages being most ventral; while of the fibres related to the body muscles, the flexor system is dorsal to the extensor system.

The more lateral and ventral longitudinal fibres of the cord are commissural between one metamere and another.

Minute Structure of the Fine Plexus.

The fine plexus is added to the cord on its ventral side in the abdominal ganglia, and is itself composed of a most complex and dense plexus of filaments which, in teased preparations, and under a high power, sometimes appear faintly moniliform. Teased preparations or sections show that the plexus varies in the fineness of its elements, and in the complexity of their arrangement in different portions. Here and there one sees in the general mass of plexus regions of the most extraordinary delicacy and density (fig. 32). These are often connected with one another by bars of equally dense material, and the masses and bars are embedded in a plexus, the elements of which are larger and more loosely arranged. The appearance is very striking in hæmatoxylin or gold-stained preparations. Into the fine plexus of the ganglion may be traced bundles of fine fibres derived from the nerve bundles, from longitudinal columns, or from the coarse plexus in which the large tubular nerve-fibres break up, and each bundle ends in one of these dense masses (fig. 32). The fine plexus is strikingly free from cells of any description. We may thus distinguish in the fine plexus what we may call "centres," each of which forms the immediate termination of a bundle of fine fibrils. Some of these are placed in immediate connection by bars of a similarly dense nature, while all are connected by the general mass of *relatively* less dense plexus.

I have already pointed out that there are regions of the ganglionic plexus to which the fibres of the various nerves may be traced. These we may speak of as the "centres" for the different nerves, and each comprises a coarse plexus of interlacing tubular fibres and a fine plexus. The former structure is merely the expression of the first branching of the large nerve fibres, while to the latter may be traced bundles of fine fibres derived (*a*) directly from the fine nerve fibres or, (*b*) from the coarse plexus. Also by means of serial sections or teasing one can follow a tubular

nerve fibre from the inter-ganglionic commissure to where it breaks up into a bundle of fibrils which are lost in the fine plexus. We must therefore look upon this structure as the place where the fibres of the nervous system ultimately communicate with one another.

Lastly, the nerve fibrils passing to the fine plexus enter it in well-defined bundles which go to histologically distinct regions, and this structural feature we may correlate with the fact that each nerve contains different groups of fibres which supply either different muscles, or regions of the sensory surface supplied by the nerve as a whole, which differ in the fact that stimulation of the one region or the other does not produce quite identical disturbances in the central nervous system.

DESCRIPTION OF FIGURES.

Part I.—*Branchippus.*

PLATES 10-13.

Fig. 1. Transverse section through ridge bounding the ventral groove in an inter-ganglionic region. The inter-ganglionic cord also shown. Oc. 4, Ob. E, ZEISS, Cam. Luc.

Fig. 2. Transverse section through mandibular region and just posterior to the mouth showing the deep post-oral groove lined by sense cells. The long posteriorly directed upper lip is seen in the lower part of the figure. Oc. 4, Ob. D, ZEISS, Cam. Luc.

Fig. 3. Transverse section through the right-hand cord between the circumoral ganglion and the brain. Oc. 2, Ob. $\frac{1}{5}$, Cam. Luc.

Fig. 4. Transverse section through the anterior commissure and just before the exit of the anterior nerve of the right-hand part of the third thoracic ganglion. Oc. 4, Ob. E, ZEISS, Cam. Luc.

Fig. 5. Transverse section of the right-hand half of the same ganglion passing through the exit of the anterior nerve. Oc. 4, Ob. E, ZEISS, Cam. Luc.

Fig. 6. Transverse section of the right-hand half of the same ganglion, and showing the posterior commissure and exit of posterior nerve. Oc. 4, Ob. E, Cam. Luc.

Fig. 7. A portion of a fibre from one of the appendage muscles, showing the termination of a nerve fibre in the protoplasmic sheath. Oc. 4, Ob. E, ZEISS, Cam. Luc.

Fig. 8. A group of ectoderm cells connected with a single (condensing) ganglion cell, which is suspended freely in the body cavity. Oc. 2, Ob $\frac{1}{5}$, Cam. Luc.

Part II.—*Astacus.*

PLATES 11–13.

Fig. 9. Dissection of the second abdominal ganglion from the ventral surface. The tissue, which lies on the ventral face of the cord and between it and the posterior sternal arteries has not been disturbed. The course of the anterior nerve on the right side is shown, and the two main branches may be traced, the anterior to the pleuron and more dorsal appendage muscles, the posterior to where it divides into two and curves down into the appendage.

Fig. 10. Dissection of that portion of the posterior nerve of the left side which runs along the ventral aspect of the flexor muscles of the abdomen.

Fig. 10A. Drawing of the same dissection from the side. The posterior nerve is still seen to be running on the surface of the flexor muscles.

Fig. 10B. The same dissection seen from the dorsal side, showing the breaking up of the branches of the posterior nerve into fan-like tufts of fibres, which pass into the extensor muscles.

Fig. 11. Transverse section through one of the anterior nerves of the second abdominal ganglion a short distance away from the ganglion. Osmic vapour, and FLEMING's fluid. Right-hand and smaller branch passes into the appendage. Oc. 2, Ob. E, ZEISS, Cam. Luc.

Fig. 12. Large nerve fibre dividing shortly after its exit from the ganglion. Fresh preparation.

Fig. 13. Transverse section through an anterior nerve of the second abdominal ganglion, before it has divided into the two main branches. Oc. 2, Ob. E, ZEISS, Cam. Luc.

Fig. 14. Transverse section through one of the posterior ventral nerves of the second abdominal ganglion close to its exit from that structure. Oc. 2, Ob. E, ZEISS, Cam. Luc.

Fig. 15. Transverse section through the posterior dorsal nerves of the second abdominal ganglion shortly after their exit from the cord. Oc. 2, Ob. E, ZEISS, Cam. Luc.

Fig. 16. Part of a transverse section through cord, showing the exit of the posterior dorsal nerves. Oc. 2, Ob. D, ZEISS, Cam. Luc.

Fig. 17. Section through the skin near the anus, showing the process of a sense cell traversing the cuticle.

Fig. 18. Transverse section through the most anterior portion of the second abdominal ganglion, showing divergence of columns. Oc. 4, Ob. A, ZEISS, tube 16·8 centims.

Fig. 19. A piece of the external sheath, or perineurium, of the ganglion isolated by teasing, and viewed from its internal surface. Oc. 2, Ob. $\frac{1}{15}$, Cam. Luc.

Fig. 20. Optical section through the external or perineurial sheath of the ganglion. Oc. 2, Ob. $\frac{1}{15}$, Cam. Luc.

Fig 21. A bundle of fibres isolated by teasing from the interganglionic cord where it enters the ganglion, showing an imperfect sheath of flattened cells. Oc. 2, Ob. $\frac{1}{15}$, Cam. Luc.

Fig. 22. Supporting or "neuroglia" cell from the ganglion. Isolated by teasing. Oc. 2, Ob. $\frac{1}{15}$, Cam. Luc.

Fig. 23. Transverse section through the inter-ganglionic cord just below the second abdominal ganglion, showing the ascending roots of the posterior dorsal nerves. From a micro-photograph.

Fig. 24. Dissection of the ganglion of the chelæ to show the relative positions of the fibres which are going to regions below, and of the fibres passing to and through the substance of the ganglion. Viewed from below.

Fig. 25. Dissection, showing the long double roots of the nerves to the chelæ and walking legs. Viewed from below.

Fig. 26. Dissection, showing the flexor muscles and the origin of the nerves of the second and third abdominal ganglia. The extensor muscles and gut have been removed, and the preparation is viewed from the dorsal surface. Thin sheets of muscle fibres arise from the belly of the great mass of the flexors in each segment, and stretch across to be joined in the middle line and above the nerve-cord by tendinous tissue. These are shown intact in Segment II., and cut in Segments V. and VI., and completely removed in Segments III. and IV.

Fig. 27. Dissection, showing the most superficial aspect of the flexor muscles. The extensor muscles of the right-hand side have been removed.

Fig. 28. Dissection, showing two myomeres of the flexor muscles. On the left, the dorsal muscle sheet, which extends outwards into the pleuron, has been removed. Diagrammatic.

Fig. 29. Dissection of the extensor system. Viewed from above.

Fig. 30. Group of nerve-cells from ganglion of chelæ, isolated by teasing from the ending in the ganglion of a bundle of fibres from the longitudinal commissures. Oc. 2, Ob. $\frac{1}{15}$.

Fig. 31. Teased preparation of "Punkt Substanz" with two nerve cells. From ganglion of chelæ. Oct. 2, Ob. E., ZEISS, Cam. Luc.

Fig. 32. Section through "Punkt Substanz," showing the entrance of a bundle of fine fibres from the posterior nerve. From section next in series to Fig. 34. Oc. 4. Ob. $\frac{1}{12}$, Cam. Luc.

Fig. 33. Section (slightly oblique) through region of entrance of anterior nerves, showing external and median centres, and the fine fibres of dorsal and ventral roots passing to them. From a micro-photograph.

Fig. 34. Section through entrance of posterior ventral nerves. Oc. 4, Ob. D, ZEISS.

Fig. 35. Longitudinal vertical section, showing the entrance of the anterior and posterior nerves into the ganglion. (Figures 32, 33, 34, and 35, are from the serial sections through the second abdominal ganglion.)

Fig. 36. Part of transverse section through the ganglion, showing the coarser and more dorsal ganglionic plexus. The nerve cells, and those of the lateral group, which are connected with the thick-sheathed tubular fibres of the interior nerves. Oc. 2, Ob. $\frac{1}{15}$, Cam. Luc.

Fig 1

Fig 2

Fig. 8

Fig 3

Fig. 6

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nerve cord

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GUT

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Fig 10

Fig. 9

Post ventral
nerve

Base of the
appendage

Fig 10b

External extensor
muscles turned up

Fig 10a.

I II III IV V

Branch to
Basal muscles
of appendage &
pleural skin

Blood vessel

Fig 11

Fig 13

Fig 12

Fig. 14

Fig 15

Fig 16

Fibres of
Post Dorsal
nerve

Group of fibres arising
from ventral portion of
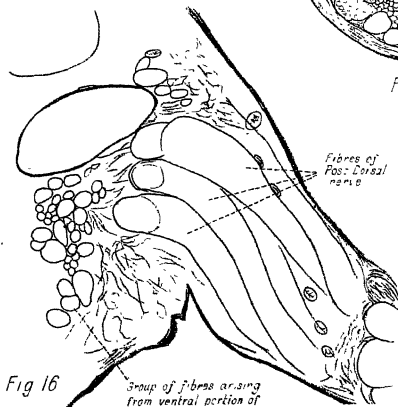
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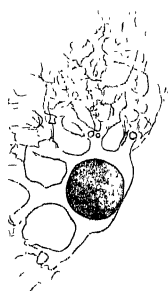
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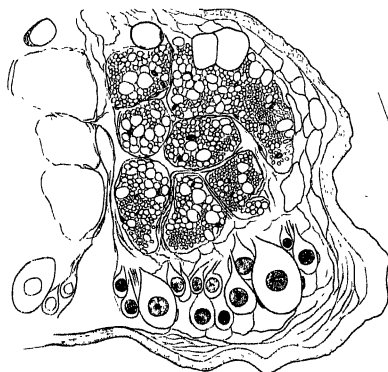
Fig 18

Fig 19

Fig 21

Fig.20.

Fig 23

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Post vent nerve

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a

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b

Fig. 35.

Fig 33.

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a

b'

c

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Fig.32

Fig 26

Fig 28

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Fig.27

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IV
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Fig 29.

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Fig 25

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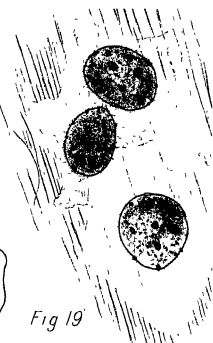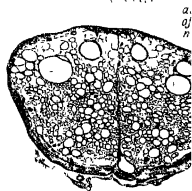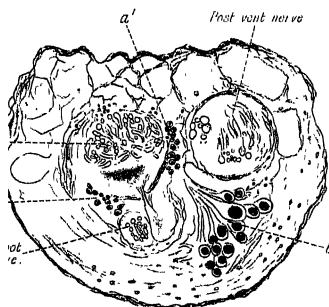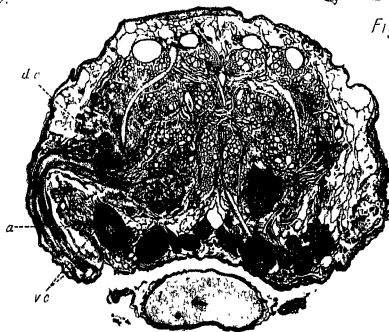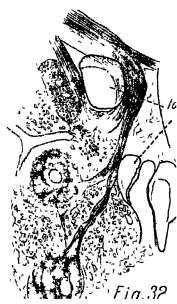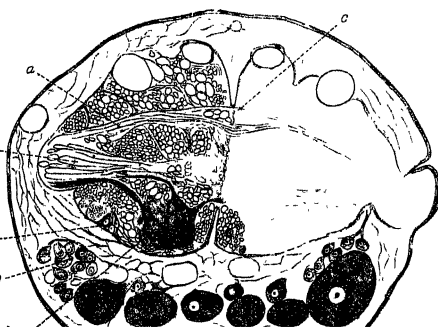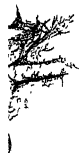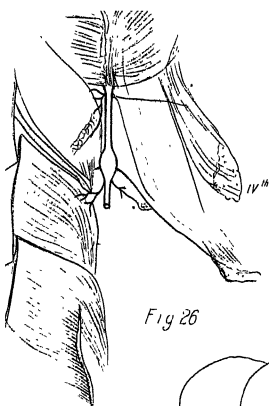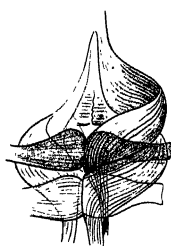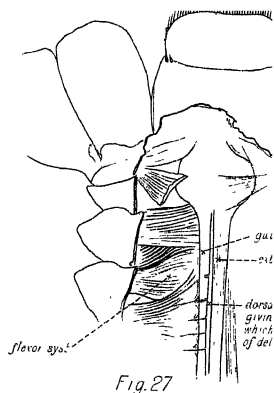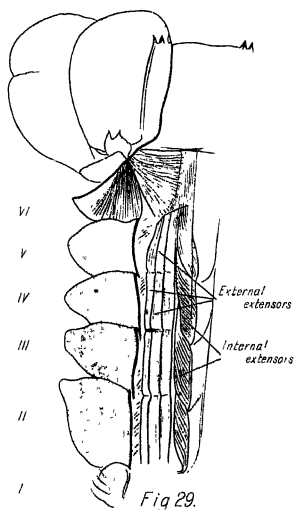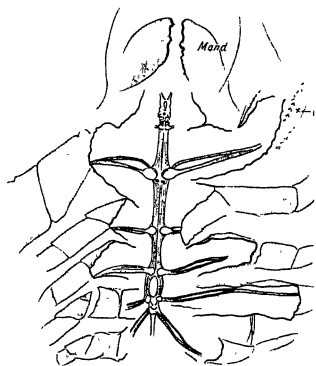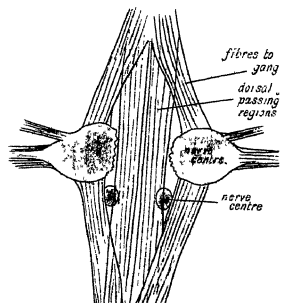
V. *On the Early Development of Cirripedia.*

By Theodore T. Groom, *B.A., B.Sc., F.G.S., late Scholar of St. John's College, Cambridge.*

Communicated by Adam Sedgwick, *F.R.S.*

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[Plates 14–28.]

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Part II.—Embryonic Development.

PART I.

INTRODUCTION.

During a short stay at Plymouth, in 1889, I was engaged in studying certain points in the anatomy of Cirripedia; finding, however, that a knowledge of the embryology was necessary in order to arrive at a complete understanding of the adult structure, I became wishful to investigate the life-history of some one member of the group. This I had an opportunity of doing at Naples, where I was appointed to occupy the Cambridge University Table at the Zoological Station for a period of six months, subsequently increased to nine. I here succeeded in obtaining a practically complete series of stages of *Balanus perforatus*, BRUGUIÈRE, as well as many stages in other members of the group.

Though a number of able observers have occupied themselves with the embryology of Cirripedes, yet, owing to lack of opportunity, and to the difficulty of obtaining complete series of developmental stages, as well as to the inherent difficulties in the subject, much remained to be done in this line. WILLEMOES-SUHM alone, with the advantages afforded by his position during the Challenger Expedition, has hitherto obtained a complete series of stages of any one form, but he failed to trace the history of the earlier stages, and in the later, limited himself to the appearance of fresh and spirit specimens, as seen without cutting sections. In fact the method of sections has been little applied to the development of Cirripedes, and not at all to the earlier stages. There is, therefore, little apology needed for an account embracing the results obtained by the employment of some of the more modern methods of embryological study.

My account of the development of *Balanus perforatus* differed in so many respects from that of previous observers on the same and other Cirripedia, that I was induced to compare the development in a number of forms, believing that the wide differences stated to exist must be due, at any rate in part, to actual differences in the development of the different forms, such as occurs in other groups in which the ontogeny of allied species and genera have been compared. This expectation was, however, only partially realised; I found, on the contrary, a great uniformity in the development of the different forms, an agreement, in fact, so close that all might be conveniently treated together. The species studied were *Balanus perforatus, Chthamalus stellatus,* POLI; *Lepas anatifera,* LINN.; *L. pectinata,* SPENGLER; and *Conchoderma virgata,* SPENGLER. I was enabled by a comparison of these forms to confirm previous work on certain points, and to establish other facts which have, in many cases, the merit of reconciling apparently discordant opinions. The present account treats of the development of these forms as far as and including the second Nauplius stage.

The early development of the Rhizocephalan genus *Peltogaster* was also investigated, but as this form showed certain differences from the others, I judged it best to reserve my account of it for a future occasion.

R

(A.) METHODS OF OBTAINING THE OVA AND EMBRYOS, AND OF EXAMINING THE OVA, EMBRYOS, AND NAUPLII.

The ova are readily obtained by cutting open the shell of the adult with a strong pair of scissors; the ovaries may then be distinguished through the membrane of the mantle cavity, or the ovigerous lamellæ may be seen lying at the bottom and sides of the cavity, free in Balanids, but attached to the *ovigerous frena* in Lepads. The lamellæ may be taken out by a pipette in the case of the smaller species, or preferably by a small section-lifter or pair of forceps; the latter are necessary in the case of the Lepads.

Placed in watch glasses the development may be watched from time to time. I found it impossible, however, to trace the whole history of the development of the Nauplii in this way, the process invariably ceasing after a certain time for want of some condition I failed to ascertain, though I tried many methods of culture. Species perhaps vary in this respect, since MÜNTER and BUCHHOLZ (22) succeeded in watching the whole course of embryonic development in one lamella of *Balanus improvisus*, DARW. In consequence of this circumstance I was compelled to examine the ova of a number of animals found at different stages, in order to complete the account. This was rendered more difficult by the fact that, as is well known, the ova of the two lamellæ of any animal are nearly always at practically the same stage of development: one can consequently search for certain stages for a long time without success. Thus, though I examined the eggs of thousands of individuals of *Balanus perforatus*, I never succeeded in obtaining an ovum, the formation of the blastoderm of which had not commenced, having thus the same experience as LANG, whose account of segmentation of this form commences when the first blastomere (his ectoderm) is already separated from the yolk (his endoderm). The mechanical labour of cutting through a solid shell, such as that of *Balanus,* becomes in such cases very tedious and occupies much time, and my best thanks are due to Dr. EISIG for supplying me with assistance in this matter. In other forms a number of stages were sometimes found in the same lamella, as in *Conchoderma virgata,* which agrees more with *Balanus improvisus* in this respect.

The methods of obtaining the Nauplii are given later.

The various stages were first of all examined by transmitted and reflected light. The embryos are sufficiently transparent, especially in some species, to show most of the details of their anatomy by transmitted light. ABBE's condenser was frequently found to be of considerable assistance for this purpose, the oblique light often being very necessary, in order to make out the cell boundaries. It was, however, always necessary to rotate the egg by moving the cover-glass along, as eggs were rarely sufficiently transparent to show the details on both sides. Some eggs, such as those of *Lepas* and *Conchoderma,* were easily detached from one another and rotated; others, such as those of *Balanus perforatus,* were often exceedingly obstinate, in consequence

of the firm character of the cementing material between the eggs. For the later embryonic stages reflected light was essential, and a condenser was found of great use in determining the boundaries of the embryonic organs.

For the histology of the embryos picro-nitric, picro-acetic, and picro-sulphuric acids and PERENYI'S fluid were good. PERENYI'S fluid was found to be a generally useful reagent, and preserved all stages well, but took longer to stain than the rest. Picro-acetic acid was also good for the embryonic stages, and, followed with borax-carmine, gave excellent results. Picro-sulphuric and picro-nitric specimens, stained with DELAFIELD'S hæmatoxylin, were also excellent, the former for the embryonic, and the latter for the free Nauplius stages.

For examination of unstained Nauplii weak osmic acid, as recommended by HOEK, was useful, as was also weak iodine, especially in the case of *Lepas* and *Conchoderma;* the former could be immediately followed by BEALE'S carmine, in the way recommended by GROBBEN (35), but some maceration inevitably followed. In addition to these, the best reagents for preserving the form were corrosive sublimate, alone or with acetic acid, PERENYI'S fluid, and chromic acid; embryos fixed by the latter reagent were, as might be expected, difficult to stain, but Nauplii from the two former fluids stained well with borax-carmine for a short time (a few minutes to a quarter of an hour).

Examination of preserved examples of the embryonic stages gave few results in consequence of the presence of the vitelline membrane, and its resistance to staining reagents.

For sections it was simply necessary to take a small piece of an ovigerous lamella preserved according to one of the above methods, imbed in paraffin, and afterwards stain on the slide. In the case of borax-carmine the staining was best done before imbedding. A large number of serial sections of all early stages of *Lepas* and *Balanus* were obtained by the aid of the Cambridge rocking microtome.

(B.) SEASONS AT WHICH THE OVA, EMBRYOS, AND NAUPLII ARE FOUND.

Before giving an account of the mode in which the eggs are laid, it may be well to state where the species may be conveniently found, and the seasons of the year at which development takes place.

The limestone rocks beneath the Marine Biological Laboratory at Plymouth, are covered in places, even considerably above high-tide mark, by small Balanids; these consist chiefly of *Chthamalus stellatus*, the eggs of which may be found in countless numbers during the months of July and August, and probably at other times.* At

* Nauplii of this species, together with those of a species of *Balanus*, were frequently obtained in large numbers at Plymouth by the use of the tow-net, during the months of February and March in 1893; by the beginning of May they had become distinctly rare; they also appear to be rare or absent at other times of the year. A few Cypris-stages, probably belonging to this form, were obtained from tubes sent from Plymouth in the middle of May, 1892.—[10/7/93.]

Newquay, in Cornwall, I found eggs also in September. At Naples the same species is common everywhere a little above the sea-level; it probably breeds here the whole year round.

Near low-tide level, at Plymouth and Naples is the larger *Balanus perforatus*, breeding also probably the whole year round at Naples, all stages being found at any time examined. Of its breeding at Plymouth I have only a single note; eggs were found at the beginning of June, the only time I looked for them.

These two species are convenient for studying the embryonic stages, the former from the ease with which all stages can be obtained, and the latter from the clear separation of blastoderm and yolk, and the distinctness with which the nuclei of the yolk-cells can be seen without special treatment.

In the case of *Balanus balanoides*, which is sparsely scattered about at Plymouth and Newquay among the *Chthamalus* (and sometimes hard to distinguish from it), but very abundant at Worm's Head, in Glamorgan, I found no eggs in July, August, or September, these observations agreeing with HOEK's statement (30) that the development lasts from November to February, or later.* I may add that I could not find this species in the Bay of Naples, a fact agreeing with DARWIN's belief that it does not extend into the Mediterranean.

For ova of *Lepas anatifera*, *L. pectinata*, and *Conchoderma virgata*, one has to rely on floating pieces of timber, ship's bottoms, &c. All stages of development were found at Naples, at any time during the year the animals were brought in.

It appears, thus, that the Cirripedes do not behave uniformly with respect to the time and duration of development. While some, such as *Balanus perforatus*, breed all the year round, others, such as *Balanus balanoides*, have one period of development, at any rate in certain localities. According to HESSE (12), *Scalpellum obliquum* breeds in the summer; I failed also, at Naples, between the months of October and May, to find eggs in *Balanus amphitrite*, though many examples were brought me, so that the breeding of this species also possibly takes place only in summer. DARWIN's supposition that Cirripedes breed several times a year (10) must probably be replaced by the statement that Cirripedes breed throughout the year, or during only a portion of it.

Some observations may be here added which it was not possible to incorporate in the table. Nauplii of *Balanus balanoides*, *B. porcatus*, *Chthamalus*, and *Verruca Strömia*, as well as Cypris-stages of *Chthamalus stellatus*, were obtained by SPENCE BATE (8), in Devonshire, during the summer.

The development of *Pollicipes polymerus* was partly worked out by NUSSBAUM, in California, during the dry season.

* Nauplii and Cypris-stages of this species appear to be abundant at Jersey, at times, during the spring, but at no other part of the year. I received examples taken by Messrs. SINEL and HORNELL in March, 1893; by the beginning of May they had again practically disappeared.—[13/7/93.]

The following table shows the months in which eggs, Nauplii, or Cypris-stages, have been obtained by myself or others. The capitals refer to the places of observation, and the numbers to the observers.

	January	February	March	April	May	June	July	August	September	October	November	December
Lepas anatifera		$N^{3,9}$	N^{9}		N^{*9}				Q^{9}	$N^{3,0}$		
„ *pectinata*	N^{9}		$N^{*}F^{*9}$	$N^{7,0}$	$N^{7,9}$				Q^{9}	N^{9}		N^{9}
„ *fascicularis*	N^{9}	N^{9}				P^{*4}	P^{*4}		Q^{9}			
Conchoderma virgata	N^{9}				N^{7}					N^{9}		N^{9}
Scalpellum vulgare				N^{9}	B^{1}	B^{1}	B^{1}	B^{1}	B^{1}	B^{1}		B^{1}
„ *obliquum*		$N^{*}P1^{9}$	$N^{*9}P1^{9}$	N^{9}	$N^{9}P1^{9}$		$P1^{9}W^{9}Q^{9}$	$P1^{9}$	Q^{9}			
Chthamalus stellatus	$N^{1,8,9}$	$N^{6,8,9}$	$N^{6,9}$	N^{9}	$N^{7,9}$	B^{1}		N^{5}	P^{9}	N^{*9}	N^{9}	N^{*9}
Chelolobia patula											N^{*9}	
Balanus perforatus						P^{9}			P^{9}			
„ *improvisus*								R^{3}	R^{3}	R^{3}	R^{3}	N^{*9}
„ *balanoides*	L^{5}	L^{5}					$o(W^{9})$	$o(P1^{9})$	$o(Q^{9})$		L^{5}	L^{5}

Place of Observation.

B. Brest (F).
F. Fontignan.
L. Leiden.
N. Naples.
P. Pacific Ocean, 32° N.
Pl. Plymouth.
Q. Newquay (Cornwall).
R. Month of R. Ryok (Pomerania).
W. Worm's Head, Glamorganshire.

* Cypris-stage observed.
o. Ova and embryos not found, though looked for at the places indicated in bracket

Observer.

1. HESSE.
2. PAGENSTECHER.
3. MÜNTER and BUCHHOLZ.
4. WILLEMOES-SUHM.
5. HOECK.
6. LANG.
7. SCHMIDTLEIN.
8. LO BIANCO.
9. GROOM.

(C.) Oviposition, Fertilization, and Formation of the Ovigerous Lamellæ.

It is well known that the ova of Cirripedes are to be found at the sides of the body in the mantle cavity, cemented together into more or less extensive lenticular sheets, termed by Darwin the "ovigerous lamellæ."

The mode in which these are formed has long been the subject of discussion, no one having observed the act of oviposition.

The ovaries were recognised as early as 1835 by Wagner (52), Mertens (53), and Martin Saint Ange (54). The latter supposed the ova to pass into the mantle cavity by means of an aperture under the carina. Darwin believed, on the other hand, that the "true ovaria" were represented by glandular organs, described by Martin Saint Ange as appendages to the stomach, and lately shown by Hoek to be really such. The oviducts, according to Darwin (9, 10), passed from the true ovaria ("enteric diverticula") in the capitulum past the base of the first pair of cirri into the peduncle, to end in the branching ovarian tubes (ovaries proper); from these the ova were believed to burst into the corium (immediately under the cuticle lining of the mantle cavity), which "resolves itself into the very delicate membrane separately enveloping each ovum, and uniting them together into two lamellæ." Upon exuviation the ova passed into the mantle cavity, while a new cuticle formed beneath.

Krohn (53), recognising with the earlier observers the ovaries proper, traced the oviduct in the reverse direction to the base of the first pair of cirri, where they opened. The ova, he supposed, made their exit through this opening.

Pagenstecher (15) did not believe with Krohn that the oviducts could be traced to the first pair of cirri, but rather that after passing up the peduncle they opened beneath the labrum.

Kossmann (56), Hoek (39), and Nussbaum (51), on the other hand, have confirmed the anatomical description of Krohn, and Nussbaum (44) has shown that the ova are at first placed at the mouth of the oviduct, becoming afterwards detached. Nussbaum also detected ova in the oviduct itself.

I was able at the beginning of the past year to complete the evidence on this question by finding an individual of *Lepas anatifera* in the act of oviposition. The ovigerous lamella of one side had been completed, and the eggs examined from time to time were seen to be developing in the usual manner; but, on the other side, the eggs had only just commenced to issue from the mouth of the oviduct, and formed a small soft rounded gelatinous mass continuous with a string of ova passing into the mouth of the oviduct, and filling the sac at the base of the first pair of cirri. The eggs of *Lepas anatifera* being of a beautiful blue colour, it was quite easy to trace the course of the oviduct, now distended with eggs, all the way from the sac at the base of the cirri through the prosoma to the ovary in the peduncle; where, not visible at first, slight pressure revealed its existence, and its course was seen to be precisely that indicated by the last-mentioned observers.

It may therefore be regarded as firmly established that the ova arising, as described by KROHN, KOSSMANN, MÜNTER and BUCHHOLZ, HOEK, and others, in the branched ovary, pass along the oviduct to the base of the first pair of cirri, and are ejected thence into the mantle cavity, where, cemented together, they form the ovigerous lamella.

As to the method of fertilization, DARWIN (10), FRITZ MÜLLER (55), and SPENCE BATE (21) have given facts showing that cross fertilization must take place in some cases. When cross fertilization fails the position of the penis seems eminently suited for self-fertilization, as inferred by MARTIN SAINT ANGE (54).

As to exactly at what moment fertilization takes place I have little fresh evidence. NUSSBAUM (44) supposed at first that it occurs in *Lepas* before the formation of the egg-sacs, and, therefore, while the eggs are still in the oviduct ; later, however (51), he supposed that the ova were fertilized as they issued from the external opening of the oviduct ; the fact that in the foregoing case of *Lepas anatifera* the ova of the completely developed lamella went on developing quite normally, while those in the small clump emitted on the other side did not develop further, though united together by the gelatinous cement and placed in exactly similar conditions, tends also to show that fertilization takes place in the mantle cavity. In all other cases (and these were fairly numerous) where I obtained eggs of Cirripedes freshly laid they had already commenced developing, and had evidently been fertilized. I am inclined to believe, therefore, that the spermatozoa do not enter the oviducts, and that fertilization takes place immediately after extrusion of the eggs, while the cementing material is still quite soft and easily penetrable.*

The ovigerous lamellæ vary much in size, colour, and number, not only in the different species, but in different individuals of the same species ; they also, as has been previously observed, in certain cases undergo definite changes in one and the same individual.

With regard to the first point, the size of the lamella is, roughly speaking, proportional to that of the individual. In all the species much variation in size and shape may be noted ; the lamella may at times be very small and tolerably thin, or may form large sheets filling up a considerable portion of the space between the body and the walls of the mantle cavity.

The newly-formed lamellæ of *Lepas* and *Conchoderma* are of a beautiful deep blue colour, and form a rounded gelatinous mass at the opening of the oviduct. As development proceeds the mass expands in all directions, and becomes a more or less extensive sheet of ova firmly cemented together ; at the same time the colour changes successively from blue into purple, red, and pink. From the colour a very good idea can be obtained of the stage of development of the ova, and though a particular tint

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* NUSSBAUM describes the material which cements the eggs together in *Pollicipes* as perforated, and suggests that the perforations serve as passages for the spermatozoa.

by no means always indicates precisely the same stage of development, this fact is exceedingly useful.

In *Scalpellum obliquum* (12), and apparently in *Pollicipes polymerus* (49), the lamellæ are yellow. In *Dichelaspis Darwinii* the ova are vermilion-red (17). In *Chthamalus stellatus*, the ova, at first flesh coloured, become bright orange-red, and the lamellæ pass gradually from this colour into paler orange and chrome-yellow. In *Balanus perforatus*, the lamellæ, at first pale yellow, become successively yellowish-grey and grey. BOVALLIUS (26) has noted a similar gradation of colour in the *Balani* he studied.

In the table, on page 130, the sizes of the ova examined are given, together with a few measurements previously published. My own measurements refer to the peri-vitelline membrane which is formed at the same corresponding period in all the species; this method of measurement was found to be the most convenient one owing to the difficulty of determining fixed points in the actual embryo. The membrane does not increase much, if at all, in size till the Nauplii are approaching maturity; any increase in growth is masked by the variations in size which occur. The vitelline membrane of the same species was repeatedly measured at different periods; the different batches (the ova of each batch being mostly at the same stage of development) often varied in average size, but quite independently of the stage reached.

From the table it is seen that the ova vary in average length from 0·11 millim. in *Balanus improvisus* to 0·31 millim. in *Balanus balanoides*, and in breadth from 0·08 millim. in *Dichelaspis* to 0·19 millim. in *Balanus balanoides*. In the species I observed myself the range was considerably less; the eggs varied in length from 0·132 millim. in *Lepas anatifera* to 0·202 millim. in *Balanus perforatus*, and in breadth from 0·084 millim. in *Chthamalus* to 0·132 millim. in *Conchoderma*. The average length in species other than those of *Balanus* ranges between 0·164 millim. and 0·17 millim., and the breadth between 0·08 millim. and 0·12 millim.*

At first, as HOEK found in *Balanus perforatus*, no increase in size in the egg takes place, but as they approach maturity the increase in size of the embryo causes elongation of the vitelline membrane in all the species I examined. In cases where the Nauplii were nearly ready to hatch, the size of the ovum was 0·25 millim. by 0·16 millim. in *Lepas anatifera;* 0·19 millim. by 0·095 millim. in *Chthamalus stellatus*, and 0·24 millim. to 0·27 millim. in length by 0·127 millim. to 0·139 millim. in breadth in *Balanus perforatus;* and 0·16 millim. to 0·17 millim. in *Balanus improvisus* (22); an increase amounting in most cases to one-half of the original length.

* DARWIN gives the size of the ova in some genera, those of *Chthamalus* and *Scalpellum* measuring respectively 0·176 millim. to 0·578 millim. In *Lepas* the length varies from 0·176 millim. to 0·226 millim.; and in *Balanus* from 0·239 millim. to 0·314 millim. But it seems probable that the latest stages are included in these measurements, and that the measurements in consequence cannot be precisely compared with those in the table. They are, however, practically within the ranges given in the tables for the corresponding genera.

Apart from this increase of size, the egg of *Lepas pectinata* is thus a little smaller than that of *Lepas anatifera*, especially in breadth, and is rather more ovate. In *Conchoderma virgata* the eggs are a little larger than those of *Lepas anatifera*, especially in breadth. In *Dichelaspis Darwinii*, according to FILIPPI, the eggs are smaller than in the foregoing genera, especially in breadth, a fact agreeing with the smaller size of the Nauplius when first hatched. The eggs of *Chthamalus* are longer than those of the foregoing genera, but as in *Dichelaspis*, narrower than in *Lepas* and *Conchoderma*. In *Balanus perforatus*, as above stated, the eggs are generally longer than in any of the foregoing, but of a breadth intermediate between that of *Lepas anatifera* and *Lepas pectinata*. The egg of *Balanus improvisus* is apparently shorter than those of the foregoing, but of the same width as *Chthamalus*; while that of *Balanus balanoides* is larger in both directions than any of the rest. In this genus, as in the others, and generally in the group, the size of the egg has a distinct relation to that of the Nauplius, but none to that of the adult.

In the table is also given the range of variation in the size of the ova. The variation is greatest in *Balanus perforatus* and *Lepas anatifera*, and least in *Lepas pectinata*.

The ova of each ovigerous lamella show a certain amount of variation, the range of which varies with the species and individual; thus five ova of *Balanus perforatus* at an early stage taken from one lamella varied in length between 0·176 millim. and 0·208 millim., while five older eggs taken from the lamella of a different individual varied in length 0·195 millim. and 0·202 millim.; in breadth the ova of the former batch varied from 0·113 millim. to 0·12 millim., while those of the latter varied from 0·094 millim. to 0·107 millim. The average length of both the batches is 0·195 millim., while the average breadths are 0·115 millim. and 0·101 millim. Longer ova are sometimes correspondingly less in width; thus, an egg 0·176 millim. in length was 0·12 millim. in breadth, while one 0·195 millim. in length, measured 0·107 millim. in breadth; generally, however, the longer ova are also broader; thus, a batch of ova averaging 0·151 millim. in length, were on the average 0·088 millim. in breadth, while a second lot, averaging 0·186 millim. in length averaged 0·11 millim. in breadth. Thus the length varies to a certain extent independently of the breadth, giving rise to differences of shape as well as size.

The average length and breadth also, as may be seen from the last example, varied in the different batches.

The amount of variation differs, apparently, in the different species, eighteen ova of *Lepas anatifera* varying between 0·132 and 0·189 millim. in length, and between 0·101 and 0·12 millim. in breadth; while seventeen ova of *Conchoderma virgata* varied between 0·158 and 0·186 millim. in length, and between 0·095 and 0·132 millim. in breadth.

Measurements of the Ova of Cirripedes in parts of a millimetre.

| | Average | | Maximum | | Minimum | | Ripe embryo. Average | | Nauplius. I. | Nauplius. II. |
	Length.	Breadth.	Length.	Breadth.	Length.	Breadth.	Length.	Breadth.	Length.	Length.
Lepas anatifera . . .	0·166	0·113	0·189	0·120	0·132	0·101	0·25	0·045	0·25	0·79
„ pectinata . . .	0·164	0·107	0·17	0·113	0·158	0·095	...	...	0·26	0·66
„ fascicularis[1] .	0·155	0·09	0·17	0·095	0·145	0·082	...	...	0·35	0·6
Conchoderma virgata	0·17	0·12	0·186	0·132	0·158	0·095	...	...	0·29	0·8
Dichelaspis Darwinii	0·16[3]	0·09[3]	...	...	...	...	...	...	0·22	0·66
Chthamalus stellatus	0·17	0·09	0·184	0·095	0·158	0·084	0·19	0·095	0·22	0·32
Balanus perforatus[4] .	0·183[3]	0·107[3]	0·202	0·120	0·139	0·082	0·24	0·031	0·28	0·46
„ improvisus[4] .	0·115	0·089	0·12	0·09	0·11	0·089	0·165	...	0·18	0·24
„ balanoides[5] .	0·3	0·175	0·31	0·19	0·29	0·16	0·3	0·15	0·36	0·45

1. WILLEMOES-SUHM gives 0·26 as the length of the ovum, but I suspect from the above measurements (which, however, were made on spirit specimens) that the 2 is a misprint for 1.

2. FILIPPI. The measurements of the Nauplii are WILLEMOES-SUHM'S.

3. HOEK gives 0·18 × 0·11 for this form; while LANG'S (32) 0·5 × 0·3 is much too great.

4. MÜNTER and BUCHHOLZ.

5. HOEK.

In Balanus improvisus and B. balanoides the average measurements given are the means of the measurements of BUCHHOLZ and HOEK respectively.

PART II.—EMBRYONIC DEVELOPMENT.

STAGE A.

The freshly laid Ovum.—Polar Bodies.—Formation of the First Blastomere.

The minute changes undergone by the Cirripede ovum during the earlier part of the embryonic period of development have been studied, in more or less detail, but often with very divergent results, by FILIPPI (17), FRITZ MÜLLER (16), MÜNTER and BUCHHOLZ (22), BOVALLIUS (26), WILLEMOES-SUHM (28), HOEK (30), LANG (32), NASONOV (40), WEISMANN and ISHIKAWA (43), SOLGER (49), and NUSSBAUM (44, 48, 51). Of these WEISMANN and ISHIKAWA, and SOLGER have confined themselves to the origin of the polar bodies. F. MÜLLER in *Tetraclita;* MÜNTER and BUCHHOLZ, BOVALLIUS, HOEK, LANG, and NASONOV in *Balanus;* and WILLEMOES-SUHM in *Lepas,* have described the segmentation as total. FILIPPI alone denied the existence of a nucleus in the "nutritive sphere." The views of these authors will be referred to again in the sequel, and I may say that my results while agreeing in many particulars with the accounts given, yet differ considerably in some respects, and I shall endeavour to show that in Cirripedes we have an extreme case of the process known as epibolic gastrulation.

Lepas anatifera.

The freshly laid ovum of *Lepas anatifera* (fig. 1) consists of a finely granular dark protoplasm, coarse and fine yolk granules, together with oil globules of various sizes. A colouring matter (soluble in spirit) gives the ovum a characteristic blue colour in many of the species. It colours both the oil globules and yolk granules : it apparently does not exist in a solid form, but as a solution which may sometimes be seen as a transparent liquid between the vitelline membrane and the embryo. Thus in one example the embryo lay in a blue solution inside the vitelline membrane. The blue colour becomes in older embryos replaced by red. As the embryo grows older the coloured yolk is used up in the formation of the almost colourless or darkly granular protoplasm, and the colour of the egg accordingly becomes fainter and fainter. This remark also applies to the ova of other genera with colouring matter of a different tint.

The protoplasm itself consists of very small refractive granules embedded in a relatively small quantity of a clear hyaline substance which can rarely be distinctly seen except at the periphery of the ovum, in the pseudopodia given off at the anterior pole, or in the vicinity of the nuclei.

The oil globules in the Cirripede ovum vary considerably in size, number, and colour. In the species under consideration, they are relatively large and few in number, and often measure as much as 0·015 millim. or more. They may be readily

recognized by blackening in osmic acid. In spirit specimens they are represented by cavities, and the circular spaces seen in thin slices are sections of these. I do not believe true vacuoles occur in the ovum of any of the Cirripedes examined.

The yolk granules (which vary in size with the species) commonly measure about 0·005 millim., but smaller sized ones occur between these, and such occasionally predominate. Under the influence of certain reagents they fuse; in other cases they appear spherical, but when well preserved and numerous they are squeezed by mutual pressure into polyhedral bodies of fairly uniform size (in the meshes of which are a number of smaller rounded granules). A peculiarity of the yolk granules is that they are hollow, being perhaps comparable in this respect to the "pseudo-cells" in *Hydra* (34). The yolk parts with picric acid and takes up staining matter with much more difficulty than the protoplasm.

A nucleus is not visible in the ovum at this stage without special treatment.

The freshly laid ovum (fig. 1) takes a definite shape (varying with the species), which is nearly ellipsoidal in *Lepas anatifera*. The egg membrane seen round all advanced embryos is not yet present : the ovum is motionless, and the constituents are uniformly scattered over it.

The first change perceptible in the egg of *Lepas* consists in the slight elongation, and in the appearance of a clear spot at the point situated most anteriorly, *i.e.*, at the apex of the blunt end of the ovum. A small dome-shaped mass of clear protoplasm elevates itself above the contour of the egg, gradually becomes conical, bacillus-shaped, pear-shaped, and finally constricts off as a clear spherical vesicle containing a few refractive granules (fig. 2), and showing on staining a nucleus with several chromatin, elements.* This is the *first polar body*. It lies generally at the apex, but sometimes a little on one side, as has been stated by NASONOV for *Balanus improvisus* (40, 41).

Sections of the ovum just before the formation of the vesicle always show a nucleus, usually very small, situated peripherally ; this may be spindle-shaped or spherical according as division is taking place or not. The direction of the spindle is generally parallel to the longer axis of the egg, but in some cases was nearly parallel to the surface and to a transverse axis. The perfectly uniform distribution of the protoplasm and yolk granules is very evident in these sections, which stain with a uniform pale tint. No radial striations of the protoplasm round the ends of the spindle could be detected during the formation of either the first or second polar body.

The first directive spindle has already been observed by WEISMANN and ISHIKAWA (43). In this, as in other cases, the germinal vesicle changed into the first directive spindle while the eggs were still in the ovary.

* The number of chromatic elements varies ; it appears to be commonly four or five, but, in some cases was, as far as I could make out, as many as ten or twelve.

As the constriction off of the first polar body takes place, the external hyaline layer of protoplasm secretes a delicate firm pellicle—the vitelline or perivitelline membrane, which encloses the ovum until the Nauplius is hatched.   It was termed the " decidua " by FILIPPI, but was regarded by NUSSBAUM in *Pollicipes* as a vitelline membrane ; the correctness of this latter designation follows from SOLGER's observations on *Balanus,* as well as from my own investigations on *Lepas, Conchoderma,* and *Chthamalus.*

The fertilization must, as NUSSBAUM supposes in *Pollicipes,* take place before the next following stage, when the second polar body is formed within the vitelline membrane, ova found at this latter stage developing normally.   I can affirm the same of *Lepas,* with the addition that ova do not appear to undergo any further changes unless they have been fertilized.   In the case of the individual spoken of under the heading of fertilization, the ovigerous lamella on one side formed a soft mass, the ova of which were giving off the second polar body ; these later formed a perfectly normal blastoderm, and had evidently been fertilized.   On the other side, the ova were only commencing to issue from the mouth of the oviduct, and had not yet given off the first polar body.   These, which were carefully observed under the same conditions as the ova on the other side, soon gave off the first polar body quite normally, and formed a vitelline membrane, but proceeded no further.

From these facts we may, I think, conclude firstly, that the entrance of the spermatozoon takes place after and independently of the formation of the first polar body, but before the formation of the second ; secondly, that fertilization probably takes place in the mantle-cavity outside the oviduct ; and thirdly, that the formation of the vitelline membrane is associated with the origin of the first polar body, and quite independent of the act of fertilization.   This fact gains in interest when it is understood that a similar membrane is also probably associated with the formation of the second polar body in the case of *Peltogaster,* the early development of which I hope to give in a future communication.

The vitelline membrane preserves more or less the shape of the ovum at the time of its formation.   In nearly all Cirripedes an anterior or larger, and a posterior or smaller pole are readily discernible from the first, though the difference between them becomes accentuated as development proceeds.   It appears thus that the anterior and posterior poles are already determined at this period.   It may be that the ovum, at first indifferent as to which of the poles of the elongated unfertilized ovum becomes anterior, only becomes orientated upon fertilization.   HOEK's observation on *Balanus balanoides* seems to point to the conclusion that fertilization takes place at the posterior end of the ovum, but even in this case it may be that the pole to be fertilized was predetermined.   I have only one observation bearing on this point ; in the unfertilized ovum of *Lepas anatifera* I could distinguish no difference between the two poles ; it must be noted, however, that the two poles show little difference in this species during any of the early embryonic phases.

The entrance of the spermatozoon has never been witnessed in Cirripedes.   HOEK

observed what were apparently remnants of spermatozoa beneath the vitelline membrane at the posterior end of the ovum in *Balanus balanoides*, though the occurrence of such beneath the membrane is somewhat puzzling, considering the mode of formation of the latter.

Sections made of ova of *Lepas anatifera* before or shortly after the formation of the first polar body show the first directive spindle or a small round nucleus with several chromatin elements. Though I searched very closely I failed to determine with certainty any body which might represent the male pronucleus, though the spermatozoon had evidently penetrated the egg. The male pronucleus must be exceedingly small and easily overlooked, otherwise it would be necessary to conclude that the fusion of the two pronuclei takes place immediately after the first polar body is formed (in which case it would be very rarely detected in ova which had given off the first polar body); but this seems improbable, though traces of a male pronucleus were never found in sections at any later phase, even in ova where the second polar body was being or had just been given off, though many ova were examined, the preservation and staining of which, as far as the chromatin elements were concerned, were excellent.

After the formation of the vitelline membrane and fertilization, the contents of the egg contract considerably (figs. 3, 44); that this is a result of fertilization follows from the fact that, while fertilized eggs contract, unfertilized ones remain uncontracted.[*]

The protoplasm now commences to undergo marked rhythmical contractions (figs. 44, 45), and after a time again swells somewhat; the clear area at the anterior end becomes amœboid, and throws out short blunt pseudopodia (fig. 3), which are as often retracted.

A blunt process, similar at first to these, gradually rises higher, and eventually becomes constricted off, and forms the *second polar body*[†] (figs. 4, 43).

Sections at this stage show either a nucleus, or the second directive spindle close to the surface (fig. 100), with its long axis parallel to that of the ovum.

The contractions of the ovum are now very marked, and give it very different appearances at different times (*cf. Lepas pectinata*, figs. 44, 45, 46). These movements are accompanied by a gradual redistribution of the material of the ovum, together with probably a production of new protoplasm from the yolk; this results in the accumulation of protoplasm in the centre of the egg, and soon in the formation of a protoplasmic part at one end, as distinct from the yolk (fig. 5 ; also *cf.* figs. 45, 83). The first attempts of the protoplasm to assume a polar position are often

[*] Among the unfertilized ova which showed diminution in size were a few that did not, and had given off a second polar body within the vitelline membrane; whether these were also unfertilized and formed an exception, or were fertilized by stray spermatozoa, I cannot say.

[†] In the case of the unfertilized ova, though no second polar body was observed, one was certainly being formed, though prevented from emerging by the close adherence of the egg to the vitelline membrane, owing to the absence of the contraction of the ovum following upon fertilization, for nearly all the sections showed the nucleus dividing again at the periphery of the ovum, precisely as in fig. 100.

somewhat crude, and the protoplasm forms an irregular readily-staining mass, some times with small outliers sharply separated from the feebly-staining yolk.

The polar bodies become pale and disintegrated, and the external one often gets washed away. The protoplasm is at last mainly collected at the anterior pole of the egg, and the yolk at the other (figs. 6, 7). The protoplasm is grey in colour and finely granular ; the yolk includes nearly all the larger granules and oil globules, but contains some protoplasm, which is centrally situated. The inferior or yolk half of the egg generally forms more than half of the whole, but often (fig. 7) the protoplasmic portion is the larger. The nucleus, which, during the period at which the ovum was undergoing contraction, was small and situated peripherally and anteriorly, and was invisible without special preparation, now becomes larger, and appears as a definite clear spot (or as a not easily staining vesicle in section), sometimes accompanied by radial striation of the protoplasm, and often visible only on pressure (fig. 7) : it simul-taneously travels towards the middle of the protoplasm. The surface separating the protoplasmic half from the yolk commonly intersects the ovum in a perfect circle, and marks off what will form the first blastomere ; though marked off from the yolk pretty sharply peripherally, the central portion of the latter has a protoplasmic mass continuous with that of the blastomere. The protoplasmic portion has almost univer-sally been regarded as ectoderm, and the yolk as endoderm. There is, however, in nearly all cases, a single nucleus in the protoplasmic portion ; the yolk at this stage is devoid of a special nucleus and is in no way comparable to an endoderm cell.

In the further development of the embryo the protoplasmic part supplies the nuclei, while the yolk provides most of the material for the protoplasm of the remainder of the ectoderm, as well as for that of the mesoderm and endoderm.

Very generally (as in all the other species) the line of separation of the protoplasm and yolk is almost accurately transverse, as described or figured by FILIPPI, MÜNTER and BUCHHOLZ, HOEK, and LANG, for other Cirripedes. In many cases, however, it lies at a small or considerable inclination to the longer axis of the ovum (fig. 10) ; we may thus get, as NUSSBAUM has observed in *Pollicipes*, all transitions from a nearly longitudinal to a transverse plane, arising by simple variation, without it being at all necessary to assume, as NUSSBAUM has done, a rotation of the plane. NUSSBAUM supposed that the plane of separation (his first cleavage plane) is parallel to the longer axis of the ovum, and later, rotates and becomes transverse. I carefully investigated the earlier stages from this point of view, and convinced myself that no rotation whatever occurs. In the position of the polar bodies my evidence is at variance with the statements of NUSSBAUM, and the facts are, I think, conclusive in favour of my own view. The polar bodies, it is admitted on all hands, are formed at the anterior pole ; if rotation occurs the polar bodies which adhere closely to the protoplasm, must, as NUSSBAUM assumes, share in the movement; and in the case of ova with oblique basal planes should show an obliquely situated polar body. Fig. 48

(*Lepas pectinata*) shows quite clearly that this is not the case ;* mostly, however, the polar bodies become rapidly lost, and I think the bodies supposed by NUSSBAUM to be polar bodies, and found by him in the furrow between the yolk and protoplasmic half, cannot be such.   I have also frequently seen cases when the wall was accurately transverse, and the polar body situated apically (figs. 6 and 7).   Lastly I have been able to watch the gradual formation of the protoplasmic half in a single ovum ; the line of junction in these cases was transverse from the first.   NUSSBAUM must then, I think, look elsewhere for support for the theory (44) he has based on this supposed rotation.

The shape of the protoplasmic portion of the egg at this stage is generally ovoid, a central plug of greater or less extent reaching into the middle of the yolk, which thus fits like a thick-bottomed bowl on to the central mass ; it is this ovoid mass which meets the periphery of the ovum in a circle.

In favourable sections the nucleus may often be seen to be dividing in a more or less longitudinal direction.   Fig. 101 shows the spindle of the segmentation-nucleus. As the first blastomere becomes constricted off, a transverse wall separates it from the yolk-cell, which receives into its central protoplasmic mass one of the daughter-nuclei of the segmentation-nucleus (fig. 102).   Traces of the spindle are often seen after the formation of the wall, and appear to penetrate the latter (figs. 102, 103).   Finally the two daughter-nuclei become quite separate (fig. 104).

Attention may be drawn to the fact that the axis of the spindle of the segmentation-nucleus is not at right angles to that of the second directive spindle ; it is quite possible, however, that rotation of the nucleus has taken place in the meantime.

STAGE B.

(A.) *Formation of the Second Blastomere.*

Lepas anatifera.

The protoplasm of the first blastomere gives rise to a portion only of the ectoderm. It has been supposed by all previous observers (BUCHHOLZ, HOEK, LANG, NASONOV, NUSSBAUM) that the ectoderm of the Cirripede arises by extension and division of this cell only.   LANG states that in *Balanus perforatus* the earlier stages are produced by division of this cell according to a definite law, while NASONOV gives in *Balanus* a definite law for the extension of the ectoderm over the whole of the yolk. For some time after studying the segmentation of the ovum I myself believed that these statements might be generally true, but signally failed in endeavouring to find the law according to which division proceeded, and before long discovered that the result was not merely owing to very considerable variation in the development, but also to a process which has been completely overlooked.   Between the stage with one

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* NUSSBAUM does not figure or describe any case of this sort.

blastoderm cell, and that with three, I could find no transitions, as also between many other stages. Allowing everything for variation I was unable to explain the facts. After much time wasted in these endeavours I found the true explanation. The second blastomere does not come from the first, but *from the yolk;* this at once gave the clue to the development, and further progress was easy.

The first external indications of the formation of the second blastomere are the gradual appearance of small granules over a definite area of the yolk to one side of the first cell (*cf.* figs. 50, 86, 88): these, from the first appearance of the patch, have a radial arrangement round a point situated near the periphery : the granules are seen to be arranged in long moniliform rows, between which are other smaller ones not showing any definite arrangement: between the rows are clear and thin lines meeting in a small central space ; at intervals along the clear lines dilatations occur. As the granular protoplasm increases, the dark central granular part of the yolk comes to the surface, and the whole body of the newly forming cell gets sharply defined peri-pherally (fig. 9); finally, the fully-formed cell may project considerably (fig. 12) above the surface, clearly defined peripherally, but not sharply marked off centrally from the yolk.

The portion of protoplasm belonging to the yolk after the formation of the first blastomere may, from the first, be situated at the surface (fig. 103), in which case the smaller spindle is obliquely directed.

The protoplasm of the cell is clearly in part a replacement of and outgrowth from the yolk, and it is very evident that some of the finely granular protoplasm is formed at the expense of the coarse granules and oil globules of the yolk, since the former at first, in many cases, increases greatly in bulk, and occupies the position of the latter, which undergoes corresponding diminution : a portion, however, often very consider-able in the case of the second blastomere, is formed from the central granular mass of the yolk, which, with the nucleus, has come to the surface. The colouring matter of the yolk thus transformed is simultaneously lost, so that a contrast in colour occurs between the greyish protoplasm and the coloured yolk.

The nucleus sometimes undergoes division, while the granular matter still occupies a central position in the yolk, and thus we get the appearance shown in fig. 8.

Sections at this stage showed that the two nuclei were certainly in some cases the result of division, so that they could not represent, as NUSSBAUM maintains, the two pronuclei ; they are very rarely seen until the separation of yolk and blastoderm is far advanced, and are preceded by a stage with a single clear nucleus, which at first is not visible. The two pronuclei are probably small ; the female pronucleus certainly is at first, and the clear spot appearing with the separation of the protoplasm is almost certainly the segmentation-nucleus. I will not, however, absolutely deny the possi-bility of such nuclei being in some cases respectively the female pronucleus, and the male pronucleus greatly grown since an earlier stage.

The nucleus of the second cell, at first sometimes scarcely discernible, as the centre

of radiation previously mentioned, grows into a clearly defined round vesicle, in the neighbourhood of which the granules for a considerable time retain their radial arrangement (fig. 14).

The second cell usually makes its appearance on one side of the yolk immediately below the first one, against which it abuts, while the free part of the circumference is part of a circle or oval. When completely formed it may occupy only a portion of the side on which it appears, or may extend down as far as the posterior pole of the yolk, and take up quite half of the latter (*cf.* fig. 48). When the protoplasm and yolk are both dark, as is often the case in *Lepas*, the first and second blastomeres are, at first sight, only to be separated with difficulty, and such appearances must, I think, have given rise to the statement of LANG that one of the daughter-cells of the first blastomere in *Balanus* grows down on one side and divides off. On careful examination and rotation of the ovum, the two are quite clearly separated, as in fig. 12.

Stages in the origin of blastomeres, in the way above described, from the yolk have evidently been observed by LANG, NUSSBAUM, and NASONOV, though the first-named stated that the appearance indicated the position of the blastopore, while both the latter would apparently regard it simply as the nucleus of the larger cell. That it does not mark the position of the blastopore is evident from the fact that it does not coincide with the point of closure of the blastoderm in any of the forms I have studied; moreover, similar cells are seen to arise in quite different positions at later stages, sometimes two or more at a time. That it is not a mere nucleus is easily seen by examination. KORSCHELT and HEIDER, in their excellent ' Lehrbuch der vergleichenden Entwicklungsgeschichte,' truly remark that, judging from NASONOV's figures, new cellular elements are formed from the yolk.

I did not succeed in ascertaining what determined the side of the ovum on which the second blastomere appeared, but that it was neither light nor gravity is evident, firstly, from the fact that light is practically cut off from the ova in many cases; and secondly, that the ova in ovigerous lamellæ of *Chthamalus*, which were sufficiently transparent to examine without disturbing their relative position, showed that the position of the cell was apparently quite capricious.

It may be noted that when the first epiblastic cell was separated from the yolk by a plane inclined towards one side, the second appeared very generally on the opposite side (figs. 10, 12; *cf.* also figs. 48, 87). This would seem to point to the conclusion already drawn by NASONOV, on observing the inclination of the plane, that the bilateral symmetry of the adult is determined at this early period. I failed, however, to confirm his statement that the polar bodies are always a little to one side.

(B.) *Growth of the Blastoderm over the Yolk.*

Lepas anatifera.

To render the following account more easily understood, it may be stated that the yolk is gradually covered by cells arising in the same way as the second blastomere,

except that the central dark mass of granular matter, at first present in the yolk, is mainly used up in the formation of the second cell, and a fresh formation of protoplasm necessary ; these cells arise generally in contiguity with the previous formed cells ; the latter, in the meantime, divide.

The blastoderm covering the yolk gradually extends from the anterior to the posterior pole ; and the complete blastoderm at the close of the stage is the result both of the origin of cells from the yolk, and of the division of such cells.

I spent much time in following the details of this process, with the result that I have been able to establish very considerable variation. It may be of interest, as indicating the purely physiological meaning of many of the processes of cell-division and disposition in Cirripede embryos, to give some of the main lines of development.

It will be convenient to refer to the plane passing through the junction of the periphery of the first blastoderm cell with the yolk as the basal plane, and to have a term for the nucleus with its surrounding protoplasm while still wholly or partly in the yolk. LANG employs RÜCKERT's term *merocyte* for such a cell in the case of meroblastic ova, and it appears to me that this term may be conveniently used in the present case.

We may, therefore, speak of the emergence of a merocyte at the surface, and term the cells which cover the yolk blastomeres, whether they arise directly from the yolk or by division of the blastomeres formed earlier.

The species whose embryonic development has been studied by myself or others are : *Lepas anatifera, L. pectinata, L. Hillii, L. fascicularis, Conchoderma virgata, Dichelaspis Darwinii, Scalpellum vulgare, Pollicipes polymerus, Chthamalus stellatus, Tetraclita porosa, Balanus perforatus, B. improvisus,* and *B. balanoides.* Other species were investigated by BOVALLIUS, but they were not distinguished, and the account given by him differs so completely from my own that I have been unable to make use of his observations ; but it is probable, from his figures and descriptions, that the development is similar to that of other forms, a probability which is reduced almost to a certainty by the fact that one of the species was *Balanus balanoides,* a form investigated also by HOEK, whose account can be readily brought into relation with my own.

In *Lepas anatifera* the basal plane may be transverse (figs. 8, 9) or oblique (figs. 10, 12).

After the emergence of the second merocyte (II.) the first blastoderm cell (I.) may divide into two cells placed symmetrically on each side of II. (fig. 14) ; or the plane of division may make any angle with that containing the nucleus of II., and the long axis of the egg (fig. 13).

A third merocyte (III.) may then emerge either to the right or left of II. (figs. 15, 16) ; while II. may sometimes divide in the same plane as I.

In a second series I. remains at first undivided, and II. divides into two cells (IIA. and IIB.) placed symmetrically on each side of I. (figs. 17, 17A). The third

merocyte (III.) then emerges generally immediately below II., while a fourth (IV.) may appear beneath I. on the side opposite to II. In the meantime I. divides transversely into a cell (IA.) near the apex and a more posteriorly situated one (IB.) situated on the side opposite II.

Other variations occur, but enough have been given to show that there is no constancy in the mode of growth of the blastoderm over the yolk.

In the early as in the later stages, the merocyte before emerging from the yolk may not uncommonly be seen to give rise by division to a second merocyte (fig. 17A), which may either produce a blastomere near the spot where it arose, or may pass to a more distant part of the yolk.

(C.) *Completion of the Blastoderm and closure of the Blastopore.*

Lepas anatifera, &c.

The exact details of the further development were not ascertained. With a larger number of cells the examination becomes increasingly difficult, both because the investigation of the number and position of the cells is very laborious, and because it is almost impossible to say in the latter stages how any given arrangement has arisen, owing to the double mode of origin of the blastomeres. As, therefore, the early stages had shown that there was so large an amount of variation in the development, I was unwilling to spend more time on the subject. The figures of the various species will give a sufficiently good idea of the further growth of the blastoderm, and a brief description, together with the explanations of the plates, is all that is necessary.

The further growth of the blastoderm takes place in precisely the same manner as in the earlier stages, *i.e.*, by the emergence of merocytes from the yolk and the division of blastoderm cells. While the merocytes are emerging as blastomeres, which generally takes place at the edge of the blastoderm, though sometimes at a distance from it, the earliest formed cells divide up further, so that the largest cells are sometimes found at the periphery of the blastoderm, and the smaller nearer the centre.

As in the earlier stages, there is a distinct tendency in the more advanced stages towards bilateral symmetry in the disposition of the cells, and the side of the ovum on which the second merocyte emerged and which took the lead in growth usually maintains, I believe throughout development, its lead. The blastoderm is finally completed in most cases on what is probably the dorsal side of the embryo at a little distance from the posterior end of the egg. The position of the blastopore, however, varies somewhat; it is generally apparently close to the position later occupied by the anus, but not uncommonly it is terminal or even sometimes ventral; in one case it was at a considerable distance in front of the end of the embryo.

With the exception of these tendencies I could detect no law in the mode of

growth of the blastoderm ; the variation is so great that the process may be said to be irregular.

The method of closure of the blastopore is difficult to observe, and was not witnessed in *Lepas anatifera*, but I succeeded in seeing it once or twice in other species (see figures of *Balanus perforatus* and *Chthamalus stellatus*).

The end of the yolk projects out at one point as a small rounded elevation (figs. 21 and 22), often of somewhat irregular outline when seen from above. A merocyte appears in the centre of this (figs. 68, 71, 94, and 127), and fills up the gap between the surrounding cells, and finally emerges from the yolk as a blastomere.

Immediately after this stage the embryo in *Balanus perforatus* consists of a single layer of cells enclosing an undivided yolk (fig. 70). All trace of the blastopore is lost, and I failed to observe any such pit as that described by NUSSBAUM in *Pollicipes* (51) at a later stage, and can confidently affirm that no such exists immediately after the closure of the blastopore.

I am unable to say how many merocytes take part in the formation of the blastoderm ; in all probability the number is variable, but not large. As the ovum is often half covered when four or five have emerged, some such number as nine or ten may not be far from the mark.

The number of cells composing the blastoderm at the time of the closure of the blastopore is also variable ; some embryos (*Lepas pectinata*) in which the latter was completely closed showed less than twenty cells, while others with an open blastopore showed a far greater number (*cf.* fig. 69 of *Balanus perforatus*).

In the vast majority of cases the yolk does not commence dividing until it is completely covered by the blastoderm, but rarely division into two takes place (*cf.* fig. 96 in *Chthamalus stellatus*).

The mode of formation of the blastoderm of all the other species of Cirripedia investigated agrees, as far as could be observed, in all essential particulars with that given for *Lepas*, but it has been thought well to mention some of the chief points under the heading of each species to illustrate the great uniformity in the general mode of development throughout the group, and, at the same time, to indicate a few points observed less perfectly in *Lepas anatifera*, as well as to call attention to slight variations not observed yet in this species.

(D.) *Stages (A), (B), and (C) in various species.*

Lepas pectinata.

In this as in all other species examined, the course of development is similar in all essential points. The ollowing points may be specially mentioned.

The egg is more ovate than that of *Lepas anatifera*, but does not differ otherwise to the eye. The colouring matter is blue, and the egg dark, the protoplasm and yolk not being readily separated. Two polar bodies were observed, the first being formed

without, and the second within the vitelline membrane ; it is worthy of note, in connection with NUSSBAUM's account of the earlier stages of segmentation, that the second polar body may be seen to be apically situated in eggs, the basal plane of which is transverse to the long axis (fig. 47).

The egg undergoes wave-like contractions (figs. 44–46), and the protoplasm collects at the broader end (fig. 46), and becomes separated off from the rest along a basal plane, which may be transverse (fig. 47), or oblique (fig. 48). This protoplasmic portion may be larger than the rest of the ovum (fig. 46), and the nucleus becomes visible after a time as a clear spot inside it (fig. 46).

The first blastomere (I.) may divide by a longitudinal constriction, or the second (II.) may do so first.   III. may appear beneath II., or on one side of it ; IV. beneath I., and V. beneath III.   Sometimes I. divides into two cells placed anteriorly and posteriorly respectively.

Conchoderma virgata.

The egg is nearly ellipsoidal in this species : the colouring matter is blue ; the protoplasm and yolk are both dark.   The protoplasm collects at one end, the yolk at the other.   The basal plane may be transverse or oblique.   I. divides by a longitudinal constriction, the axial plane passing between its two daughter-cells making any angle with that passing through the nucleus of II.   III. arises to the right or left of II., or II. divides, by a longitudinal constriction, into two cells, beneath which III. appears.   The yolk becomes covered by blastomeres emerging as in *Lepas ;* the blastopore occupies a sub-terminal (or terminal) position at the posterior end of the embryo.

Scalpellum obliquum.

In this species, according to HESSE, the colouring matter is yellow.

Pollicipes polymerus.

In this form, the development of which has been partially studied by NUSSBAUM (48, 51), the egg is ovate in shape.   The first directive spindle, according to this observer, is formed while the ovum is still in the ovary.   The first polar body is found outside, the second inside the vitelline membrane.   The egg showed waves of constriction before the protoplasm and yolk have separated.   NUSSBAUM describes two nuclei found in the ovum as the separation of the protoplasm and yolk progressed, and observing these, in some instances, to be in contact and in others remote from one another, regards them as the male and female pronuclei before and during union.   The nuclei were at first not visible even on pressure ; in other cases NUSSBAUM apparently only observed one nucleus, for he says " Einen Kern kann man

nicht leicht übersehen ; eine kleine Spindel entzieht sich fast immer an frischen, durch Einlagerungen complicirten Eiinhalt der Beobachtung." It is evident then, that the facts are capable of another interpretation, namely, that the small segmentation-nucleus gradually enlarges and divides in two in a longitudinal direction as described above in *Lepas anatifera,* and I would apply the remarks made on page 137 also to the case of *Pollicipes.*

As in the species examined by myself, the basal plane may be transverse or oblique, and NUSSBAUM's theory that rotation of the plane takes place, has been already discussed on page 135. I. may arise by a longitudinal septum. The emergence of the second merocyte has evidently been seen by NUSSBAUM, but regarded, apparently, simply as indicating the nucleus of the large cell.

Chthamalus stellatus.

In this species the egg is ovate, the colouring matter orange, the yolk granules small (0·0025 millim.), and the oil globules about as large as in *Lepas anatifera.* It seems probable that light and gravitation have nothing to do with the orientation of the poles of the Cirripede ovum, since the ova in an ovigerous lamella are orientated in all directions, as may be seen in the case of *Chthamalus* by examining the edge of a thin lamella. The wave-like contractions accompanying separation of the proto-plasm and yolk are readily observed, and the protoplasm finally collects at one end (fig. 84), the nucleus appearing as a single clear spot (fig. 84). The basal plane may be transverse (fig. 84) or oblique (fig. 85). The polar body is terminal in ova with transverse basal planes (fig. 84), (see *Pollicipes*). I. divides transversely, its plane of division making any angle with the axial plane passing through II. (fig 88), generally a right angle. III. may appear to the right or to the left of II. (fig. 89), or may arise beneath II. which has divided before I. by a longitudinal constriction into two cells placed at the same level (fig. 90).

On one occasion a newly-formed blastomere (still, however, in connection with the yolk) was seen giving off a nucleus into the yolk. Fig. 93 represents this case. The nucleus in the yolk was in this specimen connected with that of the nearest blasto-mere, or rather with that of the protoplasmic mass which will become cut off in the next blastomere, by a spindle, seen by focussing below the surface. In the vast majority of cases, however, the division of the nucleus takes place before the merocyte forms any considerable prominence upon the surface of the egg.

Fig. 94 shows the blastopore becoming closed by the emergence of a blastomere from the yolk at this point.

Tetraclita porosa.

In *Tetraclita* the basal plane is figured by F. MÜLLER as transverse, and I. is divided into two daughter-cells placed side by side.

Balanus perforatus.

The ovum in this species is ovate,* the colouring matter is greenish-brown, the oil globules small and numerous, and the yolk granules (relatively to *Lepas* and *Chthamalus*) large (0·0075 millim.) ; their outlines form a polygonal network over the surface of the yolk. The first directive spindle was observed in *Balanus* by WEISMANN and ISHIKAWA (43), though the polar body itself was not seen. The protoplasm collects at one end (fig. 49), and is well contrasted in colour with the yolk. The basal plane, generally transverse (fig. 49), may be oblique. I. may divide by a longitudinal constriction, and III. may appear to the right or left of II. (figs. 53, 54). II. may divide in the same plane as I. (fig. 54), or into an anterior and posterior cell. In other cases II. may first divide into two daughter-cells placed side by side (figs. 55, 56, 57), while III. appears immediately beneath these, or close to the posterior pole of the embryo (figs. 55, 56) ; IV. then emerges beneath I. or elsewhere, I. having in the meantime divided into a more anterior and a more posterior cell (fig. 58).

Sometimes the two daughter-cells of II. divide by a longitudinal constriction each into two cells placed side by side (fig. 57). In many cases a merocyte may be seen to divide before emerging from the yolk (figs. 53, 57, 63). Stages in the origin of the second blastomere from the yolk have evidently been observed by LANG, who regarded, however, the emerging merocyte as indicating the point of completion of the blastoderm ; that this is not so is evident from the fact that it does not coincide in position with the point of completion of the blastoderm in this form or any other examined.

Fig. 69 shows the blastopore which becomes, later, closed by an emerging merocyte (figs. 68, 71). Fig. 127 is a section passing through the blastopore at this stage.

Balanus balanoides.

The process of formation of the blastoderm in this species (see HOEK (30)) are probably quite similar to that described in *Lepas anatifera, Chthamalus stellatus, Balanus perforatus,* &c. The second polar body was seen by HOEK, but supposed by WEISMANN and ISHIKAWA to be the first. The basal plane is described and figured as transverse.

Balanus improvisus.

In this species (41, 49) the first directive spindle is formed while the ovum is still within the ovary. The first polar body is formed outside, the second inside the vitelline membrane. The basal plane here may be transverse or oblique, and II. may apparently divide by a longitudinal plane into two daughter-cells placed side by side.

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* It may be noted that the ovate shape is apparently an adaptation to that of the Nauplius shortly before hatching, it being an advantage that the vitelline membrane should offer no serious hindrance to growth.

Dichelaspis Darwinii.

In *Dichelaspis* the colouring matter, according to FILIPPI (17), is vermilion. The basal plane is figured as transverse, and the first blastomere is divided into two cells placed side by side.

FILIPPI pointed out that no nucleus was visible in the yolk (his "nutritive sphere").

Scalpellum obliquum.

The colouring matter in this form is apparently yellow (12). The basal plane is figured as oblique, and I. divides in a longitudinal plane.

Lepas, Chthamalus, Balanus.

A study of the eggs of *Lepas anatifera, Balanus perforatus*, and *Chthamalus stellatus*, by sections, confirms that of the living ova, and furnishes evidence as to the part played by the nuclei.

Sections of *Lepas*, taken at the stage in fig. 8, and slightly later, show two nuclei in the newly-formed blastoderm ; NUSSBAUM figures a similar stage in *Pollicipes*, but regards the two nuclei as the male and female pronuclei about to fuse. I have already stated my belief that these are merely the daughter-nuclei of the segmentation nucleus ; one remains as the nucleus of the first blastomere, the other passes into the yolk hemisphere (figs. 102, 103, 104), where it transforms yolk material into protoplasm ; the second merocyte formed partly in this way and partly from previously existing protoplasm, issues as the second blastomere, while the first becomes simultaneously cut off from the yolk (figs. 105 and 123).

Sections of eggs of *Balanus perforatus* show that the nucleus of the third merocyte is derived from that of the second (figs. 124*a* and 124*b*) ; the latter becomes spindle-shaped, and gives off a nucleus, which, accompanied by little or by no appreciable quantity of protoplasm, passes into the yolk, here it surrounds itself with protoplasm at the expense of the latter, and emerges generally close to the second blastomere ; after division of the nucleus (fig. 106) the latter becomes separated from the yolk by a well-defined wall (fig. 125).

The third merocyte, in similar manner, while emerging as a blastomere, divides and gives off a nucleus to the yolk, which in a similar manner gives rise to new merocytes and blastomeres.

In the later phases of segmentating eggs of *Balanus*, stages are found in which no nuclei can be detected among the yolk granules, even upon the most rigid search, but in which, as in figs. 123, 124, and 125, the newly-forming blastomere is still in communication with the yolk (fig. 126) ; in others a nucleus with a large or small quantity of protoplasm (merocyte) is found in the yolk near the edge of the blastoderm.

Such nuclei are never found beneath the cells at the anterior pole of the embryo, which are already cut off from the yolk.

These facts, together with those relating to the division of emerging merocytes, given on pages 139 and 143, indicate that as the blastoderm grows over the un-nucleated yolk, the merocytes, before or during emergence, at its edge give off in turn nuclei into, and become successively cut off from, the yolk. Once cut off they have no further connection with the yolk, which simply acts as an appendage and reservoir to the newly forming and growing cell or cells. The nucleus, at first usually small, is generally in connection with little protoplasm, but soon transforms the surrounding yolk granules and oil globules into granular protoplasm with which it clothes itself, and in this way forms a merocyte; this, at first in close proximity to the blastomere from which it has been formed, often becomes isolated in the yolk and forms a rounded protoplasmic nucleated body, with rays extending into the yolk in which it moves, and the yolk granules of which it probably devours, more or less after the manner of a phagocyte; the emergence of such merocytes gives rise to blastomeres, which in their turn give off nuclei into the yolk before becoming cut off. In fig. 125 a merocyte is seen close to one of the blastomeres, and the complete series of sections shows that the nucleus is very small and the merocyte nowhere at the surface. In figs. 126 and 127 a merocyte has come to the surface, but the cell formed has not yet been cut off from the yolk.

It is to be noted that the yolk is at no period cut off from communication with nucleated protoplasmic material in the way sometimes supposed in ova with much yolk material.

The division of the blastomeres and of the merocytes in the yolk is always accompanied by characteristic karyokinetic figures, which are often readily seen in surface views or in sections; the radial arrangement of the protoplasm is often visible long after the spindle has disappeared.*

STAGE C.

(A.) *Formation and Division of the Meso-hypoblast, and Origin from it of the Mesoblast and Hypoblast of the Nauplius.*

The further stages of development are so uniform that, with the exception of the unimportant difference mentioned on p. 149, the following account of the later stages of embryonic development, though referring chiefly to *Lepas anatifera* and *Balanus perforatus* (of which alone sections were made), will, as far as known, apply to any one of the species.

The closure of the blastopore is almost immediately followed by the division of the yolk into two pyramids or segments; the formation of the mesoblast immediately

* KRÖHENKA has recently given (51A) a few details as to the development of *Laura;* he regards the segmentation as superficial, but it may in all probability be reduced to the type described in the present paper.—[13/7/93.]

commences by the successive cutting off and sub-division of nucleated segments from the two yolk segments.

It is very rare to find stages in which the blastopore is closed and these processes have not commenced; but by prolonged search cases may be found in which the yolk is still undivided (figs. 23, 70).

In these the blastoderm consists of a single layer of cells throughout its extent, one or possibly occasionally more cells, including that filling up the blastopore, being at first still in communication with the yolk, which, as shown by sections, as yet contains no nucleus apart from that of the protoplasmic mass with which it is in communication.

In other cases the blastoderm is completely cut off from the yolk, while a single large nucleus is found in the yolk at the posterior end surrounded by a protoplasmic body sending rays between the yolk granules (figs. 70, 128).

The yolk is now in the condition of a single cell; this will give rise to the hypoblast and mesoblast; in the vast majority of cases, however, the nucleus has divided into two, and with it the whole of the yolk (figs. 24, 72, 95, 96).

It seems à priori extremely probable that the nucleus is derived from that of the merocyte which filled up the blastopore; in rare cases, however, as NUSSBAUM has already observed in *Pollicipes*, the yolk apparently divides before closure of the blastopore; it is, however, difficult to make certain of this, as the last formed cells covering the blastopore and its neighbourhood are often very low and inconspicuous, and may be readily overlooked; but if, as I think, the blastopore is really open at this stage, the single nucleus in this case must be derived from another of the cells at the posterior end of the embryo. It is not perfectly clear, therefore, whether the last point left uncovered by the blastoderm coincides in all cases with the point of origin of the endoderm and mesoderm.

Immediately after the division of the yolk the posterior ends of the two cells now composing it become more opaque, owing to the replacement of the yolk granules by polyhedral cells about the same size as those of the epiblast (fig. 97). This process may go on till quite a considerable portion of each yolk-cell is transformed into cellular material. When it is complete the anterior uninvaded portion of the yolk, still consisting of two yolk-cells, will give rise to the hypoblast, while the cells formed at the posterior end constitute the mesoblast.

Sections of *Lepas anatifera* and *Balanus perforatus* (figs. 107 to 111, 129, 130), at the stages just considered, show clearly the manner in which this is brought about. The complete external layer of cells, which may now be termed epiblast, will give rise to the ectoderm of the adult. It consists of a single layer of rounded or flattened epithelial cells, often higher anteriorly and posteriorly, the nuclei of which usually occupy about the centre of the cells; over the whole of the embryo these cells are commonly elongated and rapidly dividing by radial, but never by tangential walls. Occasionally (figs. 107, 108) cases may be seen in which the nuclei might be

supposed to be dividing along a radial plane, but careful comparison of adjacent sections shows that in this case the cells are cut more or less tangentially, and no proliferation of epiblast can be detected even upon the most rigid search.

I did not succeed in obtaining sections of embryos of *Lepas* in which no meso-blastic cells were formed, but in *Balanus*, figs. 129 and 130 represent such stages. The next stage seen is represented in figs. 107 and 108, where immediately below the epiblast are two elongated cells lying next to the two nucleated protoplasmic masses, which are continuous with the yolk. The two protoplasmic bodies are the essential parts of the two yolk-cells, and the two cells beneath are the first mesoblastic cells which have been cut off from them. This is seen from the facts that their outer boundaries are continuous with the contour of the yolk : the two cells are not coextensive with cells of the epiblast, at their junction with the latter, but are, on the contrary, coextensive with the yolk-cells, the nuclei of which may sometimes be seen dividing : they are also of a different character to the epiblast cells, inasmuch as they often contain yolk granules and oil drops, undigested remnants inherited from the yolk, and consequently the staining is less deep.

The growth of the protoplasm of the yolk-cells at the expense of the yolk con-tinues, and the two nuclei with their surrounding protoplasm pass forwards ; new mesoblast cells are cut off from the two yolk-cells, while the earlier ones divide longi-tudinally and transversely (figs. 109 to 111). The origin of the mesoblast of the yolk-cells is frequently evident even when the cells are quite numerous, owing to the original contour of the yolk being often preserved ; to the delicacy of the last formed septa, which are found next to the dividing yolk-cells; to the proximity of the nuclei of the last formed cells to those of the yolk-cells; and to the difference in the depth of staining of mesoblast and epiblast. Gradually, however, the contents of the whole mesoblast cell becomes transformed into protoplasm, and the cells become indistin-guishable except in position from those of the epiblast (fig. 111).

It may be noted that the mesoblast cells at first often seem to correspond in position on opposite sides of the septum between the two yolk-cells (fig. 110), but this correspondence soon becomes lost.

It is evident from this description that, upon closure of the blastopore, the whole yolk forms a simple cell, which will give rise to the mesoblast and hypoblast. This immediately divides into two yolk-cells, the protoplasmic portions of which are situated behind. From these two cells mesoblastic cells are successively cut off, which, like the epiblast, rapidly divide up (in a karyokinetic manner) and form a plug of mesoblast.

The mesoblast has been observed by NASONOV and by NUSSBAUM. The latter states that it is formed by the active division of superficial blastoderm cells round the edge of the blastopore before this has closed : but no such pit as that described by this observer in *Pollicipes* as existing at a time when the yolk-endoderm cells (see *posteā*) are tolerably numerous, occurs in any of the species investigated by

myself, and in none of these species did the formation of the mesoblast commence till the blastopore was closed.

My own results are more in accordance with those of NASONOV, who describes the mesoblast as originating from two symmetrically placed cells of the endoderm ; the endoderm (so-called) at this stage, however, consists of only two cells (yolk-cells), and these, as will be seen immediately, are not placed symmetrically.

The yolk, as just mentioned, is divided into two segments at this period ; the cleavage plane, in most cases where I could make certain, traverses the blastopore, or passes from a point in front of it downwards and forwards to the ventral side. It is by means of the direction of this plane that the orientation of the embryo can be first determined, and the relation of the anus to the blastopore rendered probable. It is very difficult to identify the former position of the blastopore after it has closed, but in certain cases the last-formed blastoderm cell can be recognized, either by the similarity of colour (blue in *Lepas* and *Conchoderma*) to the yolk, or by the contours of the yolk and last blastoderm cell being continuous. In rare cases division of the yolk occurs before closure of the blastopore ; in most of these cases the first cleavage plane passes through or close to the blastopore. This plane can be recognized in later stages of the embryo, and is always seen to cut the plane of bilateral symmetry at right angles. NASONOV states that it is longitudinal ; it is, I believe, never so in the species I investigated, but always inclined forwards and downwards. The inclination varies somewhat, and the plane may even appear to be nearly transverse. The yolk is thus divided into an antero-dorsal and a postero-ventral segment : each is completely cut off from the other and consists anteriorly of the usual elements of the yolk, while posteriorly is a nucleated protoplasmic section sending rays into the yolk ; there is a single large nucleus in each, close to the plane of separation, and which has evidently arisen from the single nucleus present in the preceding stage.

The yolk segments, after the separation of the mesoblast, may be termed either yolk-pyramids or yolk-endoderm cells, and will divide into a number of cells similar in character, each of which will later give rise to an endoderm cell. The two may be regarded as endoderm, but their yolk portions probably furnish nutritive material for the later growth of the mesoblast cells round the endoderm, and for the elongation of the proctodæum and stomodæum.

The two yolk-pyramids remain for a time quiescent, and do not take part in the development. The epiblast and mesoblast cells divide rapidly, the epiblast, more especially towards the poles, become more columnar, and the mesoblast forms a considerable mass at the posterior end, truncating both of the yolk pyramids (fig. 25).

The epiblast cells at the posterior end in *Lepas anatifera* (but not in any of the other species) at this and rather later stages are often rounded, while those at the anterior end have flattened tops ; the posterior area thus limited, is often strongly intersected by certain lines (figs. 25–27) ; these, however, as far as I could make out, showed no constancy ; though they sometimes doubtfully corresponded with the

Nauplius segments seen at a later stage, at other times (figs. 25–27) they were more irregular.

The further development before the appearance of any definite organs consists in a multiplication of the epiblast cells, and a growth of the mesoblast in an anterior direction on the dorsal side.

(B.) *Extension of the Mesoblast.*

The mesoblast, at first concentrated chiefly at the posterior end of the embryo (fig. 112), grows forward at the expense of the yolk, and forms a thickish plate on the dorsal side obliquely inclined from the dorsal side in front to below the terminal point behind (figs. 131, 132). It gradually extends over the embryo ; on the dorsal side it is thick, but attenuates on the sides and is scarcely represented on the ventral side and in front. The peculiar position of the mesoblast is readily brought into relation with the development of other forms when it is understood that a considerable part of it will form the muscles of the appendages of the Nauplius, the latter arising first *on the dorsal side.**

By this extension of the mesoblast along the dorsal side, the yolk comes to appear nearer to the ventral than to the dorsal side, and this has given rise, I believe, to the statement that the ectoderm becomes thickened on this side.

The yolk-pyramids, which at first were quiescent, meanwhile divide generally by planes equally inclined to both sides of the plane of symmetry ; the division takes place in each of the two pyramids ; the nucleus and daughter-nuclei† divide tangentially in the plane of symmetry repeatedly into two : the division is never radial. We thus generally get two rows of yolk-pyramids, one dorsal, the other ventral, the nuclei of which seen from above or below lie in a single line (figs. 26–28, 71–75) ; in many cases, however, the division of the cells, though anticlinal, shows no relation to the plane of symmetry, and the resulting disposition of the pyramids is irregular, as HOEK has also observed in *Balanus balanoides.*

The number of yolk-endoderm cells present at this stage, varied from about two to eight in a large number of embryos of *Balanus perforatus* and *Lepas pectinata ;* sometimes the endoderm remained divided only into two during the whole phase.

The yolk-endoderm pyramids still form a solid mass, no archenteron being yet present.

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* The sections of embryos of *Laura* (51 a, T. III., fig. 27), recently described by KNIPOVICHA, apparently also show a dorsal mesoblastic plate.—[18/7/98].

† These nuclei are usually very difficult to observe without the aid of sections in other species than *Balanus perforatus.*

STAGE D.

Formation of the Nauplius Segments.

This and the immediately following stages of the development, have been studied in more or less detail by FILIPPI, CLAPARÈDE, BUCHHOLZ, WILLEMOES-SUHM, HOEK, LANG, NASONOV, and NUSSBAUM.

Of these the observations of FILIPPI, CLAPARÈDE, WILLEMOES-SUHM, and NUSSBAUM are very brief.

FILIPPI (17) simply says that in *Dichelaspis* development begins on the ventral side.

CLAPARÈDE (14) confines himself to the statement that the segmentation of the embryo in *Lepas* is indicated by furrows.

WILLEMOES-SUHM (28) states that a groove appears, on each side of which the appendages arise. His figs. 5 and 6 do not, I am convinced, represent, as he supposes, an early stage in the segmentation of the ovum, but the division of the body, the blastoderm of which is already complete, and the endoderm well advanced in division, into the three segments of the embryo Nauplius. His fig. 7 similarly, I believe, indicates an embryo of a still earlier period, when the segmentation of the embryo had not yet commenced; the internal cells he describes are evidently the yolk pyramids, and do not, as he supposes, represent cells which ever come to the surface to form the blastoderm.

NUSSBAUM (51) states that in *Pollicipes* the anterior part of the embryo is separated by a first furrow, followed later by a second posterior to it. The appendages he describes as arising on the three segments thus formed as simple protuberances which grow towards one another on the ventral side.

More details are given by HOEK, BUCHHOLZ, LANG, and NASONOV.

BUCHHOLZ (22) gives the most detailed description, but he failed to understand the development of the first pair of antennæ.

HOEK (30) observed the division of the body into three segments, on each of which arise a pair of protuberances which grow out and form the appendages.

NASONOV (40) describes a similar stage in which he, like BUCHHOLZ, observed a median longitudinal furrow.

All the above authors have regarded the surface on which the appendages appear first as ventral.

LANG, also, though he gives few details, took the ventral surface (see his fig. 20), with the labrum and tail for the originally-thickened side of the embryo, evidently regarding the side on which the appendages appeared as ventral.

In consequence of the unusual but simple mode of origin of the appendages, much confusion has arisen as to which is ventral and which the dorsal surface, and I shall give reasons for believing that, though certain stages have been correctly described

and figured, these authors have transposed these two surfaces.   The whole course of embryonic development has been thus thrown into great confusion, and it will, I think, be simplest if I describe my own observations, pointing out at the same time where I am in accordance with previous observers.

The first indication of the external organs consists in the appearance of two perfectly straight transverse furrows on the dorsal side of the embryo (figs. 29–31). These divide the body into three divisions, the relative size of which varies somewhat ; generally the anterior one, which corresponds to the head and first segment, is largest.   The most posterior division is generally a little smaller, but may be of a size equal to, or even larger than the anterior one ; it corresponds to the third segment, and to the tail of the Nauplius together with the caudal spine.   The middle division is always smallest, and corresponds to the second Nauplius segment.

The anterior and posterior divisions are simple, and show no indication of their composition, and there is no sign of a division of any of the segments into two halves.

The furrows are complete across the dorsal surface, and pass vertically downwards on to the sides where they die out, not extending to the ventral surface.

In sections (figs. 113 and 114) the yolk-endoderm nuclei are a little larger than those of the epiblast and mesoblast, and appear scattered about in the yolk, some being near the centre, others the periphery.   The mesoblast and epiblast cells are closely associated, but the nuclei of the latter are generally elongated tangentially, rarely more or less radially.   The mesoblast nuclei are a little larger than those of the ectoderm, and are generally elongated tangentially.

Sections at the posterior end show a solid mass of epiblast and mesoblast cells ; a short distance in front there is a ring of these elements, the mesoblast being thickest dorsally, and enclosing an almost circular portion of yolk-endoderm.   In the middle of the body the mesoderm is thickest dorsally, and, extending round the sides, dies out before reaching the middle line, being represented on the ventral side only by scattered cells (fig. 114).

The mesoblast in this stage is thus shaped rather like an inverted coal-shovel, being closed on all sides except in front and ventrally, where the cells are scattered or absent.

This phase must be of short duration, as it is not often met with.

STAGE E.

Marking out of the Nauplius Appendages.   (Figs. 32–34.)

The next change consists in the appearance of a median dorsal longitudinal furrow (fig. 34), which intersects all three divisions, terminating, however, before reaching either end.   Simultaneously with this furrow two new transverse furrows appear, dividing the anterior and posterior Nauplius segments into two divisions.

The embryo is thus divided into an anterior median unpaired portion, three segments each defined by two transverse furrows, and divided into two symmetrical halves by the longitudinal furrow, and a posterior lobe.

The longitudinal furrow thus bounds internally what may be distinguished as the free ends of the appendages ($ant.^1$, $ant.^2$, $mnd.$) of the three segments.

The posterior unpaired division ($ta.$) represents the tail (thorax-abdomen) and caudal spine, the slight posterior division sometimes observed being the rudiments of the caudal forks.

Sections at this stage practically only differ from those of the preceding stage in showing the epiblast and mesoblast traversed by the dorsal longitudinal groove.

STAGE F.

Bifurcation of Second and Third Pairs of Appendages.   Origin of Labrum, Œsophagus, and Intestine.

The furrows on the dorsal side of the embryo soon become more marked, and form considerable depressions (figs. 35, 36, 76).

This phase has been accurately described by MÜNTER and BUCHHOLZ (22).

NASONOV (40) also describes the longitudinal furrow and three of the transverse ones.

NUSSBAUM (51) states that the anterior division of the body is separated off by a first furrow, a second transverse one appearing only later; but this was not so in any of the forms studied by myself.

HOEK (30) speaks of two slight constrictions on the sides of the embryo as defining the appendages, but did not apparently trace them across the embryo.

LANG (32) describes two oblique furrows as indicating the division of the embryo into three segments; he has, however, I believe, confused the stage under consideration with a later one, and his figures, 17 to 20, refer only to the later stages, the constrictions seen in which separate the labrum and tail from an intermediate region.

In all cases the side on which the furrows appear, or towards which the free ends of the appendages are directed, and on which the mesoblastic plate lies, has been not unnaturally described as ventral. I have convinced myself, however, that this side is really dorsal; firstly, from the impossibility of reconciling the appearances presented by the various stages on the former hypothesis; secondly, because the free ends of the appendages are, in all cases I have observed, or which are figured by other observers, directed towards the dorsal side till a late date; and, thirdly, because it is on the opposite side that the labrum, mouth, and nervous system arise.

I found it difficult to believe that such views as those shown in figs. 77 and 98 were

dorsal ones, but have placed it beyond all doubt by tracing the two surfaces in all the species.

The error is one easy to make, and one I did not detect until I had followed the development in detail step by step.

Since the free ends of the appendages are closely applied in the middle line, if growth in length is to take place, they must move in a longitudinal direction. They begin, therefore, to take a more oblique direction, their axes being directed not merely dorsally, but also posteriorly (figs. 35, 36, 77 to 79, 98). The first pair remain simple throughout development, while the second and third begin to bifurcate (figs. 77, 98); the free ends are divided into two before the bifurcation can be seen in surface views, dissection often being necessary to show this.

Sections of *Lepas anatifera* and *Balanus perforatus*, taken when the appendages are still quite short, show no great difference from those of the preceding stage ; the mesoblast, however, on the sides and ventral parts of the embryo is thicker (figs. 115, 133), apparently in places where the ventral muscles of the appendages will later form ; dorsally, the free ends of the appendages may be seen, together with the portion of the now free dorsal surface, with its slight mesoblastic element between them (fig. 116).*

It is hardly correct to say with NASONOV (40) that most of the mesoblast goes to form the appendages ; a not inconsiderable portion will form the muscles which, within the body, run to these appendages.

The boundaries of the mesoblastic cells are often hard to make out ; their increase apparently continues to take place at the expense of the yolk, which they more or less replace, but from which they are always sharply marked off.

Sections of *Balanus perforatus* (the embryos of the same phase in *Lepas anatifera* were unfortunately lost) show essentially the same relations, but the *œsophagus* is now seen arising apparently in connection with an epiblastic thickening not far from the anterior end and on the ventral side. It appears as a solid ingrowth projecting upwards and backwards into the yolk (*cf.* fig. 118).

No trace of the proctodæum is as yet visible.

These observations are in harmony with NASONOV's statement that in *Balanus* the stomodæum arises before the proctodæum. I could not, however, confirm his view that the œsophagus arises as a definite epiblastic invagination like that shown in his figs. 22 and 23 (No. 41). The condition figured appears to be a later one.

When the appendages have attained a certain length and degree of obliquity, and very short setæ have become visible at their tips, the ventral surface shows in side views two slight notches (fig. 37), often scarcely discernible in ventral views as narrow grooves.

* In *Laura* (51A), also, a similar ventral thickening takes place ; sections are stated to show a complete investment of the lower layer cells (mesoblast).—[13/7/93.]

The anterior of these is situated below the base of the second pair of antennæ, and marks off a slightly convex area in front, which will form the *labrum* (*lbr.*).

The second notch is short, and marks off posteriorly a triangular area which represents the *tail* (*thorax-abdomen*).

These become prominent in the latest phases of this stage (figs. 38–40, 80), the labrum becoming marked out by a parabolic furrow with two slight notches, and the triangular tail by a distinct transverse furrow; the appendages at the same time completely cover the dorsal surface of the tail.

Sections of embryos of *Lepas anatifera* and *Balanus perforatus* at this stage (figs. 117–120, 134, 135) show a further increase of the mesodermal tissue of the appendages and the muscles supplying them. At the anterior end, in front of the œsophagus, is a flat bilobed plate of cells which gives rise to the *brain* of the Nauplius. It is probably of epiblastic origin, but the embryos are so small, and the mesoblastic cells so closely applied to the very similar epiblast, that it is often quite impossible to say whether a given cell belongs to one or the other layer. The bilobed supra-œsophageal ganglionic plate is apparently continuous behind with a mass of cells (figs. 119, 134), the front part of which is traversed by the œsophagus, and which lies dorsal to, and extends into and fills up the projection forming the labrum. It is exceedingly difficult to make out the structure of this mass, but it probably contains both epiblastic and mesoblastic elements, as it occupies the site of the future labrum, sub-œsophageal ganglion, circum-œsophageal connectives, and the space dorsal to the free part of the labrum (*cf.* fig. 121); a horizontal line (fig. 119) appears to mark the future position of this space. At the sides the mass is continuous with the bases of the appendages. The œsophagus consists of a single layer of cells, arranged round an axis, and sometimes already enclosing a lumen. It has already, in some cases, the curve seen in the ripe Nauplius, sections sometimes traversing it twice. It is difficult to trace as far as the ectoderm, as it plunges into a mass of cells very similar to, and in close contact with, the cells of the epiblast, but some of which are almost certainly of mesoblastic origin. The intestine is present as a tubular mass of cells, projecting from the posterior end of the embryo far into the ventral part of the yolk, or along its under surface (figs. 120, 134). It is generally one layer of cells thick. I failed to trace it very definitely to the ectoderm, as it comes into close connection with a mass of mesoblastic cells filling up the tail (fig. 117). The extension inwards takes place, I believe, by division of the cells at the anterior end of the intestine, and by continued growth at the expense of the yolk-endoderm, which, however, almost certainly supplies only the material for growth, the cells themselves arising by division of the intestinal cells behind. Surrounding the posterior end of the intestine may be seen an accumulation of mesoblastic cells, bounding the yolk behind. These may be often seen to accompany the intestine as a thin layer (fig. 135). A similar layer surrounds the œsophagus (fig. 134). These layers, which have

apparently accompanied the œsophagus and intestine in their growth inwards, evidently represent the future circular muscles of the gut.

The yolk-endoderm cells, during stages E and F, are very inconstant in number, but usually vary between six and twelve; at the commencement of this period they are sometimes as few as two in number, and at the close are usually about twelve. The yolk-endoderm nuclei are larger and more irregular than the rounded ectoderm or mesoderm nuclei.

The ectoderm of the hinder somewhat vertically compressed part of the body is thin dorsally, ventrally, and at the sides (figs. 120 and 135). It usually separates from the endoderm and intestine ventrally, so as to leave a small space beneath the latter, occupied by reticular tissue (fig. 120).

STAGE G.

Appendages Long, with Short Setæ.   Origin of Body Cavity.

As the appendages lengthen (figs. 41 and 81) they become more and more parallel to the long axis of the body, the setæ become more distinct, and a number of those characteristic of the Nauplius when first hatched can soon be counted.

In the latter half of this period the embryos become more transparent, and the various internal organs can be distinguished, obscured only by trains of granular matter (*gr.mat.*) apparently associated with connective tissue cells.

The labrum (*lbr,*) becomes free behind and at the sides, and a considerable depression found immediately beneath it and behind its point of attachment leads into the mouth (fig. 121); this has apparently arisen by the excavation of, or splitting in, the solid mass of tissue found beneath the labrum during the last stage.

The tail (*ta.*) becomes elongated and more definitely bifid behind, where it shows two sharp points. It is separated distinctly from the shorter caudal spine (*ca.sp.*), the contour of which is continuous with that of the carapace.

The glands of the fronto-lateral horns (*frl.gl.*), not distinguishable at the beginning of the period, become defined later as two clear spaces situated in the antero-lateral angles.

The brain (*br.*) begins to be visible externally as a bilobed mass situated at the anterior end in the middle line.

Sections of *Lepas anatifera* taken at this phase (figs. 121–122e) show the mesoderm cells of the appendages as elongated spindle-shaped cells, which will form the muscles, and which extend to the dorsal and ventral sides of the body. The mesoderm of the legs and body is continuous with a portion in the labrum. The layer of cells immediately surrounding the œsophagus and intestine respectively have given rise to a single layer of spindle-shaped cells representing the muscular coats of these structures.

The nuclei of the ectoderm cells of the tail and caudal spine are now considerably enlarged, as are also the cells themselves, which are very numerous, and form a thick strand, filling up nearly the whole of the cavity of these organs.   In accordance with the rapid growth of these parts the cells have become spindle-shaped.   The spindle-shape is more marked in *Lepas* (fig. 121) than in *Balanus*.   Over the front portion of the ventral surface of the tail, however, the cells are not elongated, but simply enlarged, and constitute, at any rate in *Balanus*, the rudiment of the ventral plate seen later in the free Nauplius.

A cavity (*b.c.*), a part of which beneath the intestine is generally visible at the last stage (F), begins to form by the separation of the ectoderm and endoderm, and by irregular cavities arising by separation from one another of the differentiating cells of the partial layer of mesoderm found between the body wall and gut, or in the appendages, labrum, &c. (figs. 121*a*, 121*c*, 122).

On each side of the embryo near the anterior end are found two large comma-shaped cells closely applied to one another, and the tails of which point backwards (figs. 121*e* and 121*f*).   The protoplasm is finely granular and stains rather deeply, and the nuclei are large and granular, and provided with a single large nucleolus.   These are the glands of the fronto-lateral horns in the earliest conditions in which I have been able to recognize them.   They are closely applied to the cuticle of the fronto-lateral horns without the intervention of other cells, and are therefore in all probability greatly enlarged and specialized ectoderm cells.

The yolk-endoderm is still devoid of a cavity ; the cells have increased in number, and the nuclei are now mostly more or less peripheral.

The sub-œsophageal ganglion is now clearly visible as a bilobed mass of cells, continuous behind with the single layer of ectoderm cells of the ventral surface.   It lies above the newly-formed space, dorsal to the free part of the labrum (figs. 121 and 121*d*).   In front it is continuous with two circum-œsophageal connectives (fig. 121*c*, *c.o.c.*), themselves in intimate connection with the ectoderm.[*]

Owing to the increase in length of the appendages and general growth of the embryo the egg membrane at the end of this stage has increased much in length and often in breadth (fig. 41).

STAGE H.

Appendages with Long Setæ.   Appearance of the Nauplius Eye.   Excavation of the Mid-gut.

Finally (figs. 42, 82, 99), as the appendages attain their full length, the setæ become long and the body becomes more transparent than before ; the brain, fronto-lateral

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[*] Supra- and sub-œsophageal ganglia and circum-œsophageal connectives have been recently described in the young Nauplius of *Laura*.—[13/7/93.]

glands, alimentary canal, with its muscles, become more clearly visible; the circular muscles on the proctodæum are specially conspicuous.

The muscles of the limbs have now elongated, and form distinct muscle fibres, traversing the greatly enlarged body cavity, which now extends to all parts of the body.

The fronto-lateral horns (*frl.h.*) are clear in specimens taken out of the egg membrane. They lie parallel to the body and legs, following pretty closely the lateral margins of the carapace, and terminate posteriorly in rounded extremities a little beneath it. I regret that in consequence of the minute size of the embryos I did not succeed in observing precisely how these horns arose.

The fronto-lateral glands are now mainly filled with clear spherules of the nature of a secretion; these are closely appressed to one another, and assume thus a polyhedral shape.

The labrum acquires a shape approaching that of the free Nauplius.

The eye (*Npl.eye*) arises as two oblong yellowish-brown rectangular patches, united together in the middle line like the two halves of an open book, each half being closely applied to the lobe of the brain of the corresponding side. These gradually darken and become red; the red soon becomes, for the most part, obscured by the development of a black pigment, but a reddish tinge can often be perceived in dissected specimens at much later stages.

The granular matter is now more definitely arranged in two symmetrical masses, the greater part of which (fig. 42) lies at the sides of the gut for its whole length. Anteriorly these give off a branch passing outside the glands of the fronto-lateral horns, and another passing inwards towards the brain; the bands are united posteriorly above the intestine, and in front above the anterior end of the stomach and in front of the brain. Their nature is difficult to make out, but they apparently represent connective tissue cells, which will form bands of a peculiar tissue, which later occupy the same positions.

In ventral and lateral views a few large flattened granular cells lie outside the muscular wall of the proctodæum and stomach on their lateral walls, and meet in the median ventral line (fig. 82). These may perhaps give rise to the longitudinal muscles occupying this position later.

The yolk-endoderm cells usually number about fifteen or sixteen (or less), the additional numbers being in this and the last stage given by transverse anticlinal divisions, this process sometimes taking place tolerably symmetrically on each side of the sagittal plane. It is to be noted that the division of these cells is not accompanied by any of the karyokinetic figures such as occur in the case of both ectoderm and mesoderm cells, the process being apparently direct.

At the commencement of this stage the yolk-endoderm still forms a solid mass, but, as the time approaches for hatching, the yolk-pyramids, forming, as already stated, a single layer, commence to separate from one another at the centre (fig. 122*a*); a

cavity is thus formed, which is continuous with those formed at earlier phases in the œsophagus and intestine. The separation of the yolk-pyramids is mainly due to a diminution in size they undergo, owing, apparently, to the using up of their yolk-granules for growth of the other tissues. The successive stages in the formation of the·cavity of the mid-gut are seen in figs. 122a to e. In the final condition (figs. 122a and 122e) a single layer of cubical or tabular yolk-endoderm cells surrounds a wide cavity.

Though the cavities and walls of the œsophagus, mid-gut, and intestine are all now continuous, the three regions are still sharply marked off from one another, the yolk-endoderm cells having a large, more or less stellate, nucleus, surrounded by yolk-granules, which stain with difficulty, and the smaller cells of the fore- and hind-gut having small rounded nuclei surrounded by finely granular protoplasm, which stains readily.

The proctodæum, at its junction with the mid-gut (fig. 122d), expands to form a shallow funnel, which completes the posterior wall of the stomach. The few cells of this funnel show vertical striations, and are evidently glandular. They are very possibly already functional, and may be emitting a secretion, which acts upon the yolk-granules of the mid-gut and assists in the formation of the cavity, as the latter is often filled by a brownish plasma, presumably derived by solution of the yolk-granules. The lumen of the intestine is nearly always much larger than that of the œsophagus at this stage, and it is possible that the cells are already busy in absorbing the nutriment extracted from the yolk-granules of the mid-gut.

It may be noted that at no stage in the development of Cirripedes has an organ comparable to the " dorsal organ" of many other Crustacea been found.

The Nauplii are now ready to hatch, and, when they get free from the loosened mesh-work of egg membranes, they emerge from the mantle opening in clouds with each rhythmical movement of the adult.

PART III.—THE FREE NAUPLIUS: FIRST TWO LARVAL STAGES.

(1.) HISTORICAL SKETCH.

Various stages of the Cirripede Nauplii have been seen and described by a number of observers, but the descriptions have been in many cases incomplete, or. even erroneous; I have found it necessary, therefore, in some cases to give fresh ones.

In the following sketch I have endeavoured to trace the history of our knowledge of the earlier stages.

The Nauplii of Cirripedes were first seen in 1778 by MARTIN SLABBER (1), who observed them issuing in clouds from the shell of *Lepas fascicularis*. He gives a quite recognizable figure of the Nauplii, but regarded them as distinct forms (*Monoculus marinus*), serving as food for the Barnacle.

After the discovery by VAUGHAN THOMPSON (2) in 1830 of the Cypris stage of *Balanus*, GRAY (3) in 1833 observed the nearly ripe Nauplii of *Balanus perforatus*. He doubted THOMPSON's discovery, because of the great difference between the two larval forms, and curiously professed to see the form of the adult in the Nauplius.

BURMEISTER (4), in 1834, figured the Nauplius (and Cypris stage) of *Lepas fascicularis*; but I believe, from his description, that the Nauplii were still unhatched, and apparently not quite ready to hatch, since he states that an eye was not visible.

In 1835, THOMPSON (5) observed and figured the free Nauplii of *Conchoderma virgata* and *Lepas anatifera*. The general character of the appendages, the carapace, fronto-lateral horns, and caudal spine were described; and the peculiarities shown by the Nauplii before attaining the second stage seen, but referred to individual inability to pass on to the adult.

Of KOREN and DANIELSSEN's paper (6) on Cirripedian development I have only seen the statement (quoted by GERSTAECKER, in BRONN's 'Klassen'), that the larva of *Alepas* hatches with six legs, though it was evidently a Nauplius.

In 1843, GOODSIR (7) figured and briefly described free Nauplii seen to issue from the shell of *Balanus balanoides*. The first two stages of *Balanus balanoides* and the first stage of *Balanus tintinnabulum* were also figured. GOODSIR showed that *Balanus* must pass through two forms, viz., the Nauplius and Cypris stage.

In 1851, SPENCE BATE (8) observed and figured the Nauplii of *Balanus balanoides*, *B. porcatus*, *B. perforatus*, *Chthamalus stellatus*, and *Verruca Strömia*. He criticized GOODSIR's statement that the body showed segmentation, and observed the frontal filaments,* the labrum, and the spines on the last two pairs of limbs, and the forked abdomen, and noted that the tail was used as a rudder.

In the Monograph (1851) on the Lepadidæ (9) DARWIN gave a description of the Cirripede Nauplius, based chiefly upon the Nauplius of *Scalpellum vulgare*.

In the volume on the Balanidæ (10), which appeared in 1854, the same author gave further details based on the Nauplii of *Pyrgoma*, *Coronula*, *Platylepas*, *Alcippe*, and the other genera mentioned above, and drew attention to the uniformity in the development of the Thoracica. He observed that the Nauplius eye was composed of two halves, and also fixed the position of the anus.

MAX SCHULTZE (11), in 1853, observed in the heliotropic Nauplii of *Balanus* and *Chthamalus* the Nauplius eye, consisting of two halves, and resting directly on the brain.

KROHN (13), in 1860, figured a Balanid (*Balanus ?*) and a Lepad (*Lepas ?*) Nauplius, and correctly but briefly described and figured the brain and sub-œsophageal ganglia connected together on each side of the œsophagus in *Lepas anatifera*; he also recognized the alimentary canal, and drew attention to the fact that the anus was dorsal.

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* These were also seen and figured by SLABBER.

In 1863, CLAPARÈDE (14) described the Nauplius of *Lepas anatifera*, evidently immediately after the first moult, and gave details (though incorrect) as to the number of bristles on the appendages.

In 1864, appeared FRITZ MÜLLER's "Für DARWIN," in which the Nauplii of various groups of Entomostraca and of Prawns were compared and differences pointed out. A figure of the Nauplius of *Tetraclita porosa* is given, and the frontal filaments described as arising directly from the brain.

FILIPPI (17), in 1865, figured Nauplii of *Dichelaspis Darwinii* before and after moulting once. The form of the Nauplius is described as characteristic of the genus.

In 1866, GERSTAECKER (19) summarized the previously existing accounts of Cirripedian development (1—17), criticizing KOREN and DANIELSSEN's account for *Alepas*.

In 1869, MÜNTER and BUCHHOLZ (22) gave the first detailed account of the structure of any Cirripede Nauplius (*Balanus improvisus*) we possess. The divisions of the alimentary canal including histological details (gut muscles and cells of stomach) were given; the flexor of the tail and dorsal system of muscles to the appendages noted, and attention drawn to the differences between the Nauplii of different species of *Balanus*.

VON WILLEMOES-SUHM (28) gave in 1876 the only approximately complete account we possess of the history of any Cirripede (*Lepas fascicularis*) from the birth of the Nauplius to the fixed Barnacle. He showed the glandular function of the fronto-lateral horns.

In 1877, HOEK (30) gave a good account of the development of *Balanus balanoides*, one of the forms investigated with considerably different results by BOVALLIUS (26). Good figures of the Nauplius before and after moulting once were given. The mouth, placed by previous observers at the end of the labrum, was given its right position at the base. A lens described by a number of previous observers in connection with the Nauplius eye was shown not to exist in *Balanus*.

In the same year LANG (33) described the external character of the first two Nauplius forms of *Balanus perforatus*, and one of *Scalpellum* apparently shortly after the first moult. The characters of the appendages were given in greater detail than in previous descriptions.

SCHMIDTLEIN (37) in the same year, and LO BIANCO (46) in 1888, gave details as to the time of appearance of the Nauplii of various Cirripedes.

In 1890, GROOM and LOEB (48) gave an account of the influence of light on the movements of the Nauplii of certain Cirripedes.

In the same year NUSSBAUM (49) figured Nauplii of *Pollicipes polymerus* drawn apparently shortly after the first moult.

If the shells of the adult Cirripede are cut open, and the ovigerous lamellæ of a large number extracted, the lamellæ will be found to be at very different stages of development : some may be apparently fully developed.   If these be placed in a watch glass the Nauplii, if quite ripe, will hatch out by thousands, and can generally be collected at the two points respectively nearest to and farthest from the light ; if not far from this stage they will hatch out in limited numbers after a time. According to my experience it is only from such advanced stages, as a rule, that one can hope to get Nauplii, since once the lamellæ are taken out of the shell they appear to make only a limited amount of progress, and after a time development ceases and the eggs disintegrate, though a continuous circulation of water and air, or either alone, be kept up.   The parent appears to have some peculiar beneficial influence on the eggs.

Where a thick shell like that of *Balanus perforatus* has to be cut through the process becomes very tedious, and if the Nauplii are not wanted in large numbers they may generally be obtained by placing a considerable number of the shells in a vessel, and taking after a time by means of a pipette probes of the water from the part nearest to or farthest from the light.   The Nauplii latest hatched will generally be on the side to the light, and the earlier ones on the remote side.

In such small forms as *Chthamalus stellatus* large sheets of the cohering shells may be knocked off the rock by means of a chisel, and the lamellæ collected in great numbers ; if the ripest (generally recognizable by their paler colour) be selected a large number of Nauplii will be readily obtained.

The Lepads are not always to be obtained in such numbers as *Balanus* and *Chthamalus*, and the Nauplii are best obtained by placing full grown individuals in a large glass vessel, and noting and isolating the individuals which give off the clouds of Nauplii.   These can generally be readily recognized, as the Nauplii thus hatched out are more sluggish than those of the Balanids, and remain for a longer time in the vicinity of the parent.

Fishing with the surface-net will furnish Nauplii of *Balanus perforatus* of the first moult, and often of other moults, probably the whole year round at Naples, and in late winter and in spring the sea of part of the S.W. coast of England (Plymouth), or that around Jersey, may swarm with Cirripede larvæ (*Chthamalus, Balanus*).   In order to obtain Nauplii of *Lepas fascicularis* it is, according to WILLEMOES-SUHM (28), during the day time necessary to fish at some depth below the surface, as these. Nauplii perform the daily descent to depths characteristic of the pelagic fauna as a whole, while at night they may be obtained in vast numbers at the surface itself.

The first moult is, as a rule, speedily accomplished, as WILLEMOES-SUHM found in *Lepas fascicularis*, in fact so speedily that in this species, as well as in *Balanus perforatus*, after half an hour or so it is often difficult to find any individuals which

have not undergone it.   It appears to take place more slowly in most Lepads, but the number of batches examined was too small for generalization.

In consequence of this circumstance it is only comparatively rarely that one meets in the *Auftrieb* (result of surface-net fishing) with Nauplii which have not moulted once.

On the other hand the Nauplii of all the species kept in confinement, with exceedingly few exceptions, did not moult more than once.   I could get those of *Balanus perforatus* alone, in some cases, to moult a second time.   All my efforts to rear the Nauplii were futile.   Though they readily fed on a variety of substances, they remained for days, or even for weeks, without undergoing any change other than an unfavourable one.   I tried with large and with small quantities of water ; with water or air circulation, or both combined, or with still water ; with covered or uncovered vessels ; with vessels with a glass or sandy bottom ; in water free from or containing sea-weed ; in every case, however, without success.   The larvæ performed their daily migrations in the vessels in continually diminishing numbers, and without perceptible growth.

Most other observers have met with a similar experience ; thus Münter and Buchholz failed with *Balanus improvisus;* Max Schultze with *Balanus balanoides,* and Willemoes-Suhm with *Lepas fascicularis* at precisely the same stage.   Spence Bate makes similar statements with reference to the larvæ of the genera he studied (*Balanus, Chthamalus,* and *Verruca*).   This period may accordingly be termed a " critical " one in the life-history of Cirripedes.

In consequence, probably, of this difficulty the majority of the researches on the embryology of Cirripedes only cover some part of the period between the early stages of segmentation of the ovum and the completion of the first moult, or commence with the free or fixed Cypris stage.

I have found it convenient, therefore, to divide this subject into two sections, the first of which, forming the present paper, covers the former ground, a second will deal with the later stages.

(3.) General Structure of the Cirripede Nauplius of the First Two Stages.

The Nauplii of Cirripedes show marked differences from those of other Crustacea, yet the different forms, so far as I have examined them, or been able to ascertain from previous accounts, present great uniformity in their characters, and it will render the following comparative accounts more intelligible if I give a brief sketch of the main features which characterize the Nauplius during the first part of their period of existence (woodcuts, figs. 1 and 2).

The short and nearly colourless unsegmented body of the Nauplius is covered over a great part of the surface by a dorsal, more or less shield-shaped chitinous *carapace,* produced at the antero-lateral angles into short or long *fronto-lateral horns* (*frl.h.*), each of which is pierced after the first moult by an opening at the distal end for the

passage of the secretion of the glands situated at their base. Two smaller glands also open at two other points on the margin of the carapace in some species. The anterior margin is nearly straight, or slightly convex, the lateral margins are more rounded. The main body of the carapace is indistinctly separated posteriorly by a faint transverse line of articulation from a long movable spine, covered with spinelets, and arising from a broader base, the sides of which are continuous with the lateral convex margins of the carapace. This is the *caudal spine*, and may be regarded with DARWIN as belonging to the carapace.

Fig. 1.

Diagram showing the characters common to Cirripede Nauplii after moulting once. The tail is repre-
sented as forked, though it is simple in some genera. The sub-œsophageal ganglion is hidden by
the stomach, its posterior margin is indicated by a curved line, crossing the hinder part of the
latter. The bristles and spines are omitted from the appendages in this figure. Dorsal view.
(Lettering as in fig. 2.)

On the ventral surface, at a comparatively short distance behind the anterior margin, the very large *labrum* (*lbr.*) (with a small opening at the distal end for the passage of a glandular secretion) arises and projects downwards and backwards, making a considerable angle (fig. 151) with the body, and protecting the mouth, which lies immediately behind its base.

About two-fifths of the distance from the base of the labrum to the anterior margin spring two delicate transparent filiform organs—the *frontal filaments* (*fr.fil.*)—which are directed downwards and forwards beyond the anterior margin of the carapace.

At the sides of the base of the labrum, and in front of the mouth, spring the first pair of appendages or *antennules* (*ant.*[1]), composed of a single branch, and after the first moult very commonly directed forwards.

Fig. 2.
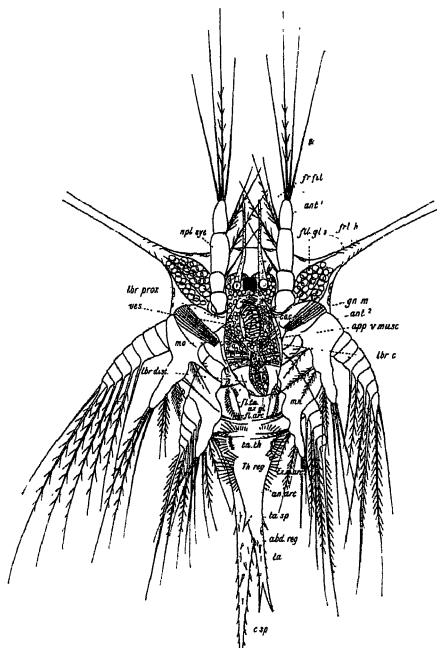
Ventral view of same. *an.*, anus; *an. arc*, anal arc; *abd. reg.*, abdominal region; *an. dil.*, dilator of anus; *ant.*[1], antennule; *ant.*[2], antenna; *app. d. musc.*, dorsal muscles to appendages; *app. v. musc.*, ventral ditto; *ax. gl.*, axial gland of labrum; *c. sp.*, caudal spine; *ect.*, ectoderm; *ex. m. arc.*, extra-maxillary arc; *fl. arc.*, flexor arc; *fl. ta.*, flexor muscle of tail; *fr. fil.*, frontal filament; *fr. fil. bs.*, base of ditto in brain; *frl. gl. n.*, nucleus of fronto-lateral gland; *frl. gl. s.* (*fil. gl. s.* in one figure), secreted spherules of fronto-lateral gland; *frl. h.*, fronto-lateral horn; *gn. m.*, muscle to gnathobase of antenna; *int.*, intestine; *lbr. c.*, cells at sides of labrum; *lbr. dist.*, distal lobe of labrum; *lbr. prox.*, proximal ditto; *mn.*, mandible; *mo.*, mouth; *npl. eye*, Nauplius eye; *œs.* (*ves.* in figures), œsophagus; *st.*, stomach; *sub-œs. g.* (*sub. ves. g.* in figure), sub-œsophageal ganglion (seen through the labrum); *ta.*, tail (thorax abdomen); *ta. sp.*, tail spine; *ta. th.*, tail-thickening or rudiment of "ventral plate."

Opposite the front corners of the mouth spring the second pair of appendages or *antennæ* (*ant.*[2]), the largest of the three pairs.

These are followed immediately by the third pair of appendages or *mandibles* (*mnd.*), which, like the second, are biramous.

The character of these appendages is remarkably uniform throughout the group, and will be given later on.

Behind the mouth is found a median region, traversed or bounded by characteristic bands of hairs, generally directed forwards, and forming with those of the labrum a sieve, which apparently serves to retain particles of any size which have found their way beneath the labrum. This region may be termed the *setose region;* it occupies the position of the future gnathites.

Posterior again to this is the *tail* (*ta.*) of the Nauplius, consisting of two regions, an anterior of short, truncated conical shape, extending from the setose region to the level of the two strong spines (*ta.sp.*), and corresponding in position with the future thorax : this may be termed the *thoracic region* (*th.reg.*) ; while the region marked anteriorly by the spines and forming a long spine covered with spinelets and generally bifurcated towards the end may be termed the *abdominal region*, since it marks out the region of the future abdomen and caudal appendages of the Cypris-stage.

Until after the completion of the first moult the tail and caudal spine are both telescoped.

The *mouth* (*mo.*), situated behind and at the base of the labrum, leads into a short *œsophagus* (*œs.*), which passes at first slightly downwards and forwards, and then curving round, at first upwards and forwards, and then upwards into the stomach. The *stomach* (*st.*) is a large simple rounded sac occupying the centre of the body. The *intestine* (*int.*) is straight, and about as long as the stomach, from the posterior end of which it passes to the anus. Like the œsophagus, it undergoes incessant peristaltic contractions. The *anus* (*an.*) lies at the base of the tail and on its dorsal side, that is between the tail and caudal spine.

The *nervous system* consists of, firstly, a lobed bilaterally symmetrical *brain* (*br.*), the halves of which are closely applied together in the middle line ; secondly, two stout *circum-œsophageal connectives* (*c.o.c.*) ; and thirdly, a *sub-œsophageal ganglion* (*sub-œs.g.*).

The *Nauplius eye* (*Npl.eye*) is black, and consists of two halves, sometimes separated, but generally closely applied together in the middle line, each resting on one of the halves of the brain.

On each side of this the brain gives direct origin to a frontal filament.

At the base of the fronto-lateral horns are two large spindle-shaped glandular organs—the *fronto-lateral glands* (*frl.gl.*)—each consisting, in all cases, of two closely applied unicellular glands attached on one hand to a point not far from the middle of the carapace above the sides of the stomach, and on the other opening into the cavity of the fronto-lateral horns.

Two other glands—the *lateral glands* (*lat.gl.*)—(probably also unicellular), usually

much smaller, occur in some genera in the posterior part of the body, where they open externally by minute pores on the sides of the carapace.

The body cavity (using the term in the descriptive sense of a cavity included within the body-walls and surrounding the viscera) is spacious, and extends into every portion of the body. Its main section is bounded posteriorly by a delicate sheet of tissue (*an.dil.*) which separates off the cavities in the caudal spine and tail, and probably serves to dilate the anus. The body-cavity is transversed also by strands of branched and anastomosing connective tissue cells and contractile fibres, extending, at fairly frequent intervals, between the alimentary canal and body wall.

Distinctly *striated muscles* (*app.d.musc.* and *app.v.musc.*) also pass from the median dorsal and median ventral lines above and below the stomach to the appendages ; others are limited to portions of the appendages.

With the exception of those of the walls of the alimentary canal the only other muscles to be found are the *flexors of the tail* (*fl.ta.*), a pair of muscles which pass from the carapace (close to the attachment of the fronto-lateral glands) downwards and backwards to the sides of the setose region.

A special accumulation of mesoderm cells at the sides of the labrum will be referred to later.

On part of the ventral surface of the setose region in most of the genera, and in *Balanus* also in the thoracic and abdominal region, a special accumulation and disposition of the ectoderm cells (*ta.th.*) occurs on each side, the cells being at the same time peculiarly modified ; the fate of these cells will be followed hereafter.

(4.) DETAILED STRUCTURE OF THE NAUPLII OF STAGES 1 AND 2.

Stage 1. *Between the Periods of Hatching and of the First Moult.*

The newly hatched Nauplii are active little creatures (figs. 140, 153, 161, 164, and 167), much smaller than after the completion of the first moult.

The dimensions in the different species are as follows: *Lepas anatifera*, 0·25 millim.: *L. pectinata*, 0·26 millim.; *L. fascicularis*, 0·35 millim. (28); *Conchoderma virgata*, 0·29 millim.; *Dichelaspis Darwinii* and *Chthamalus stellatus*, 0·22 millim.; *Balanus perforatus*, 0·28 millim.; *B. improvisus*, 0·18 millim. to 0·19 millim. (22); and *B. balanoides*, 0·36 millim. to 0·37 millim. (30).

The young Nauplius is more or less pear-shaped, or sometimes conical; broadest in *Balanus*, and narrowest in *Dichelaspis*.

The telescoping of the tail (*ta.*) and the posterior spine (*c.sp.*), and the direction of the fronto-lateral horns, are the most striking features of the Nauplius at this stage. The dorsal views show the telescoping of the dorsal spine, which lies invaginated within the body and extends as far as a point above the centre of the stomach. The

point alone projects from the tube. The tail is similarly invaginated and projects as one or two points according as the abdomen is simple or forked.

The long or short fronto-lateral horns are directed backwards, extending in *Lepas* and *Conchoderma* nearly to the end of the body. Their ends are not open, as in all the later stages, but always closed and perfectly rounded. They are often seen to contain spherules of a glandular secretion.

The frontal filaments (*fr.fil.*), present in all the later Nauplius stages as well as in the Cypris stage, are absent, as some earlier observers have pointed out. I failed to find the stump-like rudiments described by LANG as representing them in *Balanus perforatus*, but could sometimes observe the filaments lying under the cuticle about to be cast off.

The appendages are only indistinctly articulated, and the hairs, spines, and teeth, which are present in considerable number, are all simple, *i.e.*, not plumose or furnished with secondary spines. In consequence of the indistinct articulation of the joints, and of the fineness and shortness of the hairs, both the number of joints and hairs were exceedingly difficult to ascertain, but the disposition given in the table on p. 182 certainly holds good for *Balanus perforatus* and, probably (from the perfect similarity in the appendages of all the species after the first moult), for the rest of the species. The agreement of Stage 1 with Stage 2 is seen from the table to be very close, the former having rather fewer joints and hairs.

The labrum has not yet acquired its definite shape, the distal lobe having rounded angles, and the teeth and hairs characteristic of it at later stages are absent.

The hairs on the setose region of later stages are also absent.

The granular tissue is plentiful and still prevents the internal organs being seen perfectly. It runs in fairly defined trains at the sides of the alimentary canal, above the anterior part of the stomach and posterior part of the brain, and in front of and behind the fronto-lateral glands.

In other respects than those just mentioned, the whole organization is essentially the same as after the first moult, when the structure can be made out more clearly.

Figures and descriptions of Cirripede Nauplii at Stage 1 have been already given for *Conchoderma virgata*, by THOMPSON (5); *Balanus tintinnabulum*, by GOODSIR (7); *B. balanoides*, by GOODSIR and SPENCE BATE (8); *B. perforatus*, by BATE and LANG (33); *B. porcatus, Verruca Strömia*, and *Chthamalus stellatus*, by BATE; *Dichelaspis Darwinii*, by FILIPPI (17); *Balanus improvisus*, by MÜNTER and BUCHHOLZ (22); *Lepas fascicularis*, by WILLEMOES-SUHM (28); and *Pollicipes polymerus*, by NUSSBAUM (51).

Few of these, however, are sufficiently detailed to be of much value for comparative purposes; the best figures are those of *Balanus balanoides*, by HOEK; *B. improvisus*, by MÜNTER and BUCHHOLZ; and *Lepas fascicularis*, by WILLEMOES-SUHM.

Stage 2. (*Between the Periods of the First and Second Moults.*)   Figs. 210, 211,
220–222, 225–227, 232, 235, 238.

(A.) *The First Moult.*

Nauplii after the first moult have been figured for *Lepas anatifera,* by MARTIN
SLABBER (1) and CLAPARÉDE (14) ; for *Lepas anatifera* and *Conchoderma virgata,*
by VAUGHAN THOMPSON (5) ; for *Balanus balanoides,* by GOODSIR, SPENCE BATE, and
HOEK (7, 8, 30) ; for *Verruca Strömia* and *Chthamalus stellatus,* by SPENCE BATE ; for
Scalpellum vulgare, by DARWIN and LANG (10, 32) ; for *Tetraclita porosa,* by FRITZ
MÜLLER (16) ; for *Balanus perforatus,* by MÜNTER and BUCHHOLZ (22) ; for *Lepas
fascicularis,* by WILLEMOES-SUHM, (28) ; for *Balanus perforatus,* by LANG (32) ; and
for *Pollicipes polymerus,* by NUSSBAUM (51). Of these the best figures are those
of BUCHHOLZ, WILLEMOES-SUHM, and HOEK ; those of LANG and SPENCE BATE are
also valuable.* None of these authors, however, have given detailed accounts of
the structure, and the descriptions are not always correct ; nor have any com-
parative accounts been given of the Nauplii of the different genera and species. I
have in the following account attempted to partially fill up this gap in our know-
ledge by means of a careful study of the Nauplii as seen whole, dissected, or in serial
sections.

The increased transparency of the larvæ, due to the disappearance of the granular
material, and to the expansion of parts crowded together beneath the cuticle of the
Nauplius of the first stage, renders the Nauplius much more favourable for examina-
tion than before, while the transparency of the tissues beneath the carapace renders
them better for study than the later Nauplius stages.

The time which elapses between birth and the first moult appears to vary according
to conditions, and with the species. I have practically no recorded observations on
this question, but according to the best of my recollection the Nauplii of *Balanus
perforatus* at Naples underwent their moult within the first half-hour or so ;
Chthamalus stellatus at Plymouth and *Lepas pectinata* at Naples were similar, and
rapidly attained the full development of the second stage ; while *Lepas anatifera* and
Conchoderma virgata, whilst possibly undergoing the moult with equal rapidity
(though I did not observe this), were often many days before the tail, caudal spine,
and appendages showed their full development, though sometimes the change was
more rapid ; I suspect that in their natural condition the change is generally more
rapid, as the movements of the comparatively sluggish Nauplii seemed to be retarded
by a thin coating of viscid material which formed at the bottom of the dishes in
which they were placed. Mere confinement under artificial conditions may also, very
possibly, have some effect on the rate of change.

SPENCE BATE found in all the species he examined (Balanids) that the moult took

* I regret that I have been unable to see HESSE's second paper on the development of *Scalpellum* (25)
in which figures, which may include the stage under consideration, have been given.

place on the second day : in *Bulanus improvisus,* according to BUCHHOLZ, the moult took place at latest on the third day. GOODSIR found in *Balanus balanoides,* also examined by BATE, that the moult took place after eight days. It would appear possible that the duration of the first stage depends partly on latitude, but not altogether, since *Chthamalus stellatus,* one of the species examined by SPENCE BATE, and stated to moult on the second day, sometimes, as stated above, moults very shortly after hatching, so that if the observations are correct other conditions than latitude must come into consideration.

The changes accompanying the first moult were briefly characterized by WILLEMOES-SUHM in *Lepas fascicularis,* but have generally been ill-understood, and have given rise to some misconceptions. They are simple, however, and need but brief description.

When the time for moulting arrives the cuticle of the first stage is burst and thrown off. The Nauplius issues from a large gap at the anterior end of the cuticle ; the water entering this gap probably distends the cuticle, and assists in the process of exuviation which is apparently brought about by ordinary locomotive movements.

The tail and posterior spine, hitherto telescoped far within the body, become evaginated ; the hairs formed inside those of the previous stage were likewise telescoped, and as they become evaginated the secondary hairs with which they are furnished, and which at first must lie parallel to the sides of the tube inside which they were formed, become visible. Thus the tail, caudal spine, and bristles often appear jointed, the joint occurring where the invaginated portion begins (figs. 165 and 166). CLAPARÈDE has already seen this telescoping of the hairs in the Nauplii of *Lepas anatifera,* and LANG in those of *Scalpellum vulgare,* while WILLEMOES-SUHM observed it in the case of the tail and caudal spine of *Lepas fascicularis,* and HOEK in the case of the caudal spine in *Balanus balanoides.**

This appearance, however, has given rise to the statement (33) that the caudal spine in *Balanus perforatus* consists of two segments.

WILLEMOES-SUHM has given the same explanation as myself.

NUSSBAUM, on the other hand, observed Nauplii of *Pollicipes* sometimes with a long, and at other times with a short caudal spine ; the shorter, he states (p. 29), is only the longer drawn within the body by a muscle, the spine being protruded or retracted at will. This statement is apparently based on inference, as one can readily perceive in *Lepas* and *Conchoderma* that the length of the tail and caudal spine depends on age ; the supposed muscle is simply a strand of somewhat elongated ectoderm and connective tissue cells.†

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* GROBBEN (65) and CLAUS (61) figure the caudal bristles of *Branchipus* as arising from a kind of sac apparently in the same way as the setæ of Chætopods : I suspect, however, that they are simply telescoped as in the above-mentioned cases, and that the invaginated sacs have no essential resemblance to the permanent sacs of Chætopods with their special muscles. The telescoping of the tail and caudal spine suggests comparison with the method of formation of organs in Hexapod insects by "imaginal discs."

† The "peculiar chitinous structures" figured by KNIPOVICHA (51A) in the young Nauplius of *Laura*

The gradual evagination of these structures, and the curving forward of the fronto-lateral horns form the most marked changes the Nauplius undergoes at this moult ; the frontal filaments appear at once, as also do the bristles and teeth of the setose region and labrum, and the carapace becomes larger; the internal organization clearer.

The changes resulting in this transformation are so marked that LANG assumed two or three moults to be necessary for their completion in *Balanus perforatus*. In *Lepas* and *Conchoderma*, where the contrast is much greater owing to the length of the tail and caudal spine, seeing all stages of the transition, I thought that not less than four or five moults had taken place, and spent some time in fruitless endeavours to find constant differences between the different phases.

Several examples of these stages are figured (figs. 155, 165, 166) in *Lepas anatifera* and *Conchoderma virgata*.

LANG's figure of *Scalpellum* also probably represents a Nauplius at this transitional stage, as also do THOMPSON's figures of *Conchoderma virgata*, CLAPARÈDE's of *Lepas anatifera* and FILIPPI's of *Dichelaspis Darwinii*.

(B.) *Size of the Nauplii.*

The Nauplii, after moulting once, attain a size much greater than that of the first stage.

The lengths (taken in the species I examined from the front margin to the end of the caudal spine) are as follows:—*Balanus improvisus*, 0·24 millim. (22) ; *Balanus balanoides*, 0·45 millim. (30) ; *Balanus perforatus*, 0·46 millim. ; *Chthamalus stellatus*, 0·32 millim. ; *Dichelaspis Darwinii*, 0·66 millim. (17) ; *Lepas fascicularis*, 0·6 millim. (28) ; *Lepas pectinata*, 0·66 millim. ; *Lepas anatifera*, 0·79 millim., and *Conchoderma virgata*, 0·8 millim.

The Nauplii of each species vary somewhat in absolute length and breadth, and the length and breadth vary independently, giving slight differences in shape, but the number of cases noted is too small to be worth recording.

(C.) *The Carapace.*

The carapace is characteristic in most of the genera.

It is essentially an expansion of the dorsal side of the body in anterior, lateral, and posterior directions, the cuticle on the dorsal side of which is somewhat thicker than over the rest of the body.

It is shield-shaped and shallow in *Lepas* and *Conchoderma* (figs. 156, 157, 162), and has its anterior angles produced into two long and slender delicately striated horns (*frl.h.*) ; these are directed slightly downwards and forwards, and terminate in

may, perhaps, represent the invaginated caudal spine and adjoining folded cuticle just before a moult.—[13/7/93.]

an aperture bounded by slight anterior and posterior spine-shaped edges, between which are dorsal and ventral V-shaped gaps. The posterior margin of the carapace is produced into a long median caudal spine (*c.sp.*), articulated at the base and covered with slender spinelets.

In *Dichelaspis Darwinii* (fig. 168) the carapace is triangular; the caudal spine about the same length as in *Lepas* and *Conchoderma*, but the horns much shorter.

In *Balanus* the carapace apparently varies somewhat in shape. In *Balanus perforatus* (figs. 141, 142) it is rather broadly shield-shaped and distinctly convex, with a somewhat convex anterior edge, short fronto-lateral horns, and two short blunt teeth on its lateral margin just where it passes into the broad-based caudal spine; the latter is much shorter than in the foregoing genera, and is covered with minute spines.

In *Balanus improvisus* (22) the frontal horns are relatively about as long as in *Balanus perforatus*, but the carapace is apparently more triangular, and the caudal spine relatively shorter.

In *Balanus balanoides* the caudal spine and fronto-lateral horns are still shorter, and farthest removed from the Lepad type.

In *Chthamalus stellatus* (figs. 149–151) the carapace approaches a circular or ill-marked hexagonal form; the anterior and posterior margins are straight and parallel; the fronto-lateral horns as short as in *Balanus perforatus*; the lateral margins show two slight bulges at about two-thirds of the length of the carapace from the fronto-lateral horns; these mark the openings of two large lateral glands; the caudal spine has a very broad base with toothed margins, and is covered with rather short and strong secondary spines. In this species the thickening of the cuticle extends anteriorly on to the ventral side, and ends in an abrupt transverse line just in front of the frontal filaments.

In *Tetraclita porosa* [relying on FRITZ MÜLLER'S figure (16)], the carapace is also nearly circular, and in general appearance much like that of *Chthamalus stellatus*.

In *Scalpellum vulgare*, according to the figures of DARWIN and LANG, the carapace is very broadly shield-shaped, with bulging sides and fairly short horns; the caudal spine is short in the figures, but it appears possible that this may be due to its being telescoped, especially as LANG describes the hairs on the appendages as telescoped.

In *Pollicipes polymerus*, as figured by NUSSBAUM, the carapace is very similar to that of *Scalpellum*, having prominent sides, fairly short horns, and a short caudal spine; how long the latter is does not appear from the description or figure, since it is not stated whether, in NUSSBAUM'S Plate 2, fig. 3, it is protruded or not; probably it is retracted.

In *Verruca Strömia*, judging from SPENCE BATE'S figures (though these are apparently somewhat diagrammatic), as that author has pointed out, the caudal spine is much longer than in *Balanus balanoides*. It is apparently about as long relatively as that of *Balanus perforatus*, which has the longest spine of all the Balanid species hitherto observed. The shape of the carapace closely approaches that of *Balanus*,

and the fronto-lateral horns are apparently relatively about as long as in *Balanus perforatus* or *B. improvisus.*

It is thus evident that the carapace varies from a broad and convex shield-shaped or almost circular structure with short horns and caudal spine to a shallow more or less triangular structure with long horns and posterior spine.

Its surface is quite smooth in *Balanus perforatus,* but in *Conchoderma, Lepas,* and *Chthamalus* it shows a fine reticular structure, which I at first imagined was due to the outlines of minute ectoderm cells below it till I perceived that the ectoderm cells were too scattered to produce such marking. In cases where decomposition of the animal has set in the markings are lost, though delicate chitinous hairs remain. The markings are evidently not in the chitin, but belong, in some way not perfectly clear to me, to the more decomposable tissues immediately beneath. The meshes of the network are fairly large in *Chthamalus,* but very small in *Lepas* and *Conchoderma;* in the latter two forms its distribution gives rise to a tolerably definite pattern formed by a series of intersecting curves, the main lines of which run in definite systems, which have, in many cases, a marked relation to the points of attachment of fibres passing from the carapace to the walls of the alimentary canal.

The cuticle over the whole of the body is everywhere underlaid by the ectoderm, a layer of protoplasm (except where the muscles are attached), which has a finely granular appearance and is generally thin, but locally thickened in the neighbourhood of the large scattered nuclei, with their distinct nucleoli. At the sides of the carapace is a band pretty definitely marked off from the rest along which the ectoderm cells have a vesicular appearance.

At the base of the fronto-lateral horns are situated in all the species examined a pair of large glands—the fronto-lateral glands (*frl.gl.*)—which are probably to be regarded as specially developed ectoderm cells, though the embryological evidence on this point is not complete.

The gland has the same structure in all the species. It consists of two greatly developed cells which have strongly refractive chitinous walls; these completely invest each cell except at the distal ends, where the chitinous sac ends in an irregular margin within the tubular horns. The two chitinous sacs which are respectively anterior and inferior, and posterior and superior, are closely applied together for the whole of their length, and together form a spindle-shaped organ extending from a point on the right or left side of the lower surface of the carapace above the sides of the stomach : the main and distal portion of each sac is either empty or filled with spherules of a transparent secretion, which are generally so numerous as to be pressed together into polyhedral bodies (*frl.gl.s.*). When found outside the horns these drops of secretion are spherical.

A considerably smaller portion of the proximal end of each sac is occupied by finely granular protoplasm, with a large nucleus (seen in *Lepas, Conchoderma,* and *Balanus*) with a reticular meshwork of deeply staining threads and particles (fig. 138).

The protoplasm, as it is traced away from the nucleus, becomes filled by droplets of the secretion, which finally merge together to form the larger spherules filling the sac.

The posterior of the sacs was often found to be empty in *Lepas*.

The lumen of the horns is commonly partially interrupted in *Balanus* and *Chthamalus* (figs. 141, 142, 149) by structures looking like spines or hairs. It must have been this structure which led DARWIN to believe that the axis of the horn was occupied by a plumose process, regarded by him as corresponding to the prehensile antennules of the Cypris-stage. They are commonly attached only to one side, and are, I suspect, caused by the solidification of the peripheral part of the secreted spherules while still within the tube, and the bursting of these caused by the pressure of the secretion behind ; the partitions, at all events, closely resemble the walls of the spherules of secretion filling up the cavity of the glandular sacs.

The fronto-lateral glands are, accordingly, a pair of large unicellular and uni-nucleated glands.*

Posterior to these, in *Balanus perforatus* and *Chthamalus stellatus,* are a pair of similar but rather smaller glands (figs. 141, 149, *lat.gl.*), pretty definitely marked off from the rest of the tissues, but without the strong refractive investment of the fronto-lateral glands. These are attached to the carapace close to the point of inser-tion of the fronto-lateral glands ; they pass backwards and outwards to a point about one-third of the length of the margin of the carapace in front of the commencement of the caudal spine, and open externally by a pair of apertures very prominent in *Chthamalus,* and situated on a part of the margin bulging out beyond the rest (fig. 150).

These are the *lateral glands :* they are absent at this stage in *Lepas* and *Concho-derma*.

In all three forms a third structure occurs (*d.b.* in figs. 156, 157, 162), which, on account of its resemblance to the glands of the labrum to be described shortly, I am disposed to regard as glandular.

It consists of a short cord of very granular or vesicular tissue (*d.b.*), arising from the dorsal surface of the investment of the brain close behind the eye and passing upwards and forwards to the carapace. Its substance is distinct from that of the brain, and there seems no reason to infer any intimate connection between the two. In later stages it is clearly seen to be unicellular.

(D.) *The Labrum.*

The labrum (*lbr.*), though always large, varies considerably in relative size and form, and in details of structure, in the different genera. It is characteristic in *Lepas, Dichelaspis, Chthamalus,* and *Balanus,* and probably in other genera ; in *Conchoderma,* however, in agreement with the close affinity of this genus

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* They appear to be essentially similar in structure to the two pairs of glands described and figured by GROBBEN in the tail region of the Copepod *Cetochilus*.

with *Lepas*, it is indistinguishable from the labrum of that species.   In other genera it is unknown to me, and the published figures, with, perhaps, the exception of *Verruca*, are not sufficiently good to rely upon.

It is very large in *Lepas* and *Conchoderma*, where it extends to the posterior end of the body.

In *Dichelaspis* it is as long, but more slender and pointed.   In *Balanus* it is relatively broad and short.

It consists in the above-mentioned genera of a proximal (*lbr.prox.*) and a distal lobe (*lbr.dist.*).   Both are furnished with a row of long hairs on the upper surface (that turned towards the ventral side of the post-oral region) : these follow the margins for the greater part of the length of the labrum, but proximally turn inwards and converge towards the mouth.

The proximal lobe is ovate in shape in *Lepas*, *Conchoderma*, *Dichelaspis*, and *Chthamalus*, but broader and shorter in *Chthamalus* than in the others.   In *Balanus* it is also broad and short, but its distal end is furnished with two small lateral lobes which give it a characteristic shape.

The distal lobe, though smaller than the proximal, is large and pentagonal in *Lepas* and *Conchoderma*, resembling in shape the section of a rather flat-topped haystack, and is provided with one large perforated median and two smaller lateral teeth on its expanded distal margin.   In *Dichelaspis* it is narrow, parallel-sided, and pointed at the end.   In *Chthamalus* it is large and parabolic, and supplied with two or three distinct median and two lateral teeth, together with three or four smaller ones between these on each side.   In *Balanus* the median lobe is similar in shape though smaller, and supplied with two lateral teeth, one on each side.

In *Verruca*, relying on Spence Bate's figure, the basal lobe of the labrum is large and ovate ; the distal lobe small and pentagonal, and resembles *Lepas* and *Conchoderma* in the disposition of the teeth (one median and two lateral), but agrees more closely with *Dichelaspis* in the prominence of the median part bearing the unpaired tooth.

. ˙ The labrum has sometimes been stated to be movable, but I never observed this, and satisfied myself that no muscles pass to it with the exception of short ones from the œsophagus.

The labrum is longer on its lower than on its upper side, the lower being attached immediately behind the eye, and the upper at about the level of the point of attachment of the antennæ.

The axis of the labrum is occupied by a peculiar gland (*ax.gl.*) confined to it, and opening at its distal end.

This occurs probably in the Nauplii of all the Thoracic Cirripedes ; I have observed it in *Lepas*, *Conchoderma*, *Dichelaspis*, *Chthamalus*, and *Balanus*.   It is of different nature to the fronto-lateral and lateral glands.   Though essentially similar in all these it varies somewhat in the different groups.

It is simplest in *Lepas anatifera, L. pectinata,* and *Conchoderma,* for which forms one description will suffice. It is readily visible in all specimens of these genera as a narrow cord of granular or vesicular matter running from the base of the labrum to its end. It is this, and not a simple groove as BALFOUR supposed, which has been taken by so many observers for the œsophagus.

The gland on careful examination is seen to be composite, and to consist of four elongated and probably unicellular glands, two of which, forming a pair, are attached to the sides of the base of the labrum some distance in front of the mouth (figs. 158, 159), and two lying close together, forming a central strand inserted immediately below and in front of the mouth; all three strands unite in the middle line, while still within the proximal lobe of the labrum, but, maintaining their independence, pass towards the end of the distal lobe of the labrum (within which, not far from their termination, they may become slightly expanded and diminish) to the central tooth by an aperture, on the summit of which they open to the exterior.

The contents vary somewhat, but generally consist of deeply-staining, coarse granules of uniform size, but in many cases a different appearance is caused by the presence of a number of vacuoles.

I observed in some cases one, and, as far as I could ascertain definitely, only one nucleus for each cell, generally placed near the proximal end.

In the remaining forms examined the contents of the glands consisted of finely granulated vacuolated material.

In *Dichelaspis Darwinii* (fig. 168), as far as I could make out from mounted specimens, the form of the gland approaches that of *Lepas* and *Conchoderma,* except that its distal end is expanded into a spindle-shaped body, filling up the greater part of the distal lobe of the labrum. One or more of the large vacuoles are generally present.

In *Chthamalus stellatus,* in which the labrum was only investigated in spirit specimens, I could only make out the distal portion of the gland; this is swollen (fig. 150) in a marked manner at the tip, as in the case of *Balanus.*

In *Balanus perforatus* (figs. 139*a*, 142, and 145) the gland consists of four pear-shaped cells which meet together at the distal end of the labrum, but in accordance with the diminution in length of the labrum the gland is shorter owing to the absence of the part corresponding to the proximal portion in *Lepas.* The nuclei are large and distinct, and furnished with a distinct intranuclear network, and generally resemble those of the fronto-lateral glands, but are smaller. The glands are here distinctly seen to be unicellular. A delicate axial fibre (figs. 136*d* and 145, *ax.gl.fi.*), apparently of the nature of connective tissue, runs from a small group of cells situated immediately in front of the mouth to the distal end of the axial gland, serving, perhaps, as a support for the gland cells.*

* These glands apparently correspond with those figured but not described by CLAUS ('61) in the labrum of the Nauplius of *Branchipus.*

Immediately behind the labrum, as HOEK alone has stated for *Balanus balanoides*, and BALFOUR supposed in *Lepas fascicularis*, is the mouth, with a strong chitinous margin, which excavates with its lower half the base of the labrum. This side is also deepened by two grooves bounding a small median lobe between them, while the chitin is thinner and less conspicuous on the upper margin.

(E.) *The Setose Region.*

The setose region (figs. 144, 152, 160, and 163) presents certain characters common to the whole group, but the details, though constant in the species, are often distinctive of the genera.

Passing down the sides of the region from the mouth in an elegant lyre-shaped curve in *Lepas, Conchoderma, Chthamalus,* and *Balanus* is a band of long hairs (which may be double) bending in towards the middle line and immediately beneath the ventral insertions of the flexor muscles. These may be termed the flexor arcs (*fl.arc*).

In front of the flexor arcs, disconnected in *Chthamalus*, united together by two slight ventral loops in *Balanus*, and by a distinct band in *Lepas* and *Conchoderma* (where it is immediately followed by a second slight transverse band), a band extends on each side from about half way along the flexor arcs towards the ventral side of the region; these may be termed the *anterior arcs (ant.arc)*. In the normal position of the parts they lie immediately above the labrum, forming with the hairs of that organ a sieve, preventing the escape of large particles from beneath it.

In *Balanus* and *Chthamalus* there is also a median group of hairs on the region above the base of the labrum and not far behind the mouth.

Behind the flexor arc are two short curved lateral bands, between the anterior ends of which are two slight curved bands in *Lepas, Conchoderma,* and *Balanus,* apparently represented by a single transverse band in *Chthamalus*. The lateral bands may be termed the *extra-maxillary arcs (ex.mx.arc)*, and the smaller bands the *pre-maxillary bands (pmx.bd.)*.

Posterior and nearly in a line with the former are two bands of long bristles passing obliquely upwards to unite on the dorsal side of the tail behind the anus: these are the *anal arcs (an.arc)*; they belong properly to the thoracic region.

In *Balanus* the extra-maxillary bands are only slightly developed.

In *Lepas* and *Conchoderma* a *maxillary arc* runs from the point of junction of the extra-maxillary and anal arcs.

These terms are used to facilitate descriptions, and because some knowledge of the area will be required in discussing the morphology of the gnathites.

(F.) *The Tail (Thorax-abdomen).*

The tail (*ta.*) varies considerably in length and appearance in the different genera, and to some extent in the species.

In *Lepas anatifera, L. pectinata, L. fascicularis* (28), probably in *L. anserifera* (5), and in *Dichelaspis*, it is long and simple, and covered with fairly long secondary spines.

In all the examples of *Conchoderma* investigated it was similar, but bifurcated at the tip.

In *Verruca* (21) it is apparently similar to *Conchoderma*, but shorter.

In *Chthamalus stellatus* it is short and the spines few.

In *Balanus* the secondary spines are much shorter. In *Balanus perforatus* the tail is rather short, in *B. improvisus* (22) it is still shorter, and in *B. balanoides* (30) apparently very short.

At some distance behind the base of the tail in all the species are a pair of very strong ventrally and laterally directed spines, with toothed postero-ventral and antero-dorsal edges, the teeth being much more numerous and marked on the ventral side in most species. The teeth of the posterior margins are continuous across the middle line.

These spines mark the commencement of the abdomen, and, as will be seen later, correspond partly to the caudal appendages of the Cypris stage. The posterior end of the head region is marked off by the maxillary arc.

The thorax occurring between these limits is marked laterally by the anal arc, and is generally provided with fewer and shorter spines than the caudal or abdominal region.

(G.) *The Appendages.*

Fortunately the appendages, which might be supposed to need the longest description in a comparative account, show no important difference in any of the species I have examined.

It is not a little remarkable that with the exception of the presence of some short simple hairs in *Lepas*, which are absent in *Balanus*, and of slight differences in the relative proportions of the parts, the structure of the appendages is exactly the same in each of the species. There are the same number of joints on corresponding branches or basal pieces, and the same number of bristles, spines, or teeth on each of the joints; these processes are numerous, and present great variety in structure, but the differences are repeated in each species, so that there are precisely the same number of simple bristles, plumose bristles, simple spines, plumose spines, teeth, &c., in the Nauplius of every species at this stage.

This is true of such different forms as *Lepas anatifera, L. pectinata, Conchoderma virgata, Chthamalus stellatus, Balanus perforatus,* and probably all the rest of the Thoracica.

Few who have studied Cirripedian development have attempted to minutely characterize the appendages, and a careful comparison of the species has given results, which, while they agree perfectly together, only partially agree with the

statements of those who have. This, however, is not difficult to understand considering the difficulty of counting numerous hairs on such minute forms, and I must say that it was only a perception of the very close agreement between all the species that saved me, as I believe, from error myself. For I had only to see a difference in two of my sketches to discover upon re-examination of the object that a mistake had been made in one or other.

In *Balanus improvisus*, the characters of the appendages, as given by BUCHHOLZ, much resemble that given below; in *Balanus balanoides* (30) the description agrees even more closely, while in LANG'S description of the Nauplius of *Balanus perforatus*, which is the only account in which minute details are given, the agreement is almost perfect, but, still not quite exact, but as this is one of the species I examined this discordance must disappear.

In describing the appendages it will be convenient to recognize the following— *hairs*, small and simple ; *bristles*, much stronger ; *plumose bristles*, with hairs on one or both sides; *spines* and *plumose spines* much stiffer ; *conical processes* and *teeth* still stronger ; and the *gnathobase* as a movable biting piece.

It will abbreviate description to state that all the bristles, spines, &c., at this stage, arise on the ventral sides of the appendages, with the exception of the line of simple hairs on the dorsal sides of the second and third pairs of appendages ; and that the bristles and spines curve inwards towards the centre of the ventral surface. It is also to be noted that the secondary hairs on the bristles are given off in the *dorso-ventral* plane on one or both sides.

The antennules or first pair of Nauplius appendages are a pair of uniramous, elongated appendages, generally directed downwards and forwards, parallel to the long axis of the body, but during motion moved to a position at right angles to this.

They consist of four joints, of which the first or basal is of truncated conical shape and devoid of hairs, the narrow end fitting by means of a flexible membrane into a circular opening in the body wall situated opposite the extreme anterior end of the labrum, in the angle between that organ and the lobes of the brain ; they are thus distinctly pre-oral in position ; the remaining joints are cylindrical; the second bears a simple bristle at the distal end ; the third is much the largest, and gives off about its middle a plumose bristle, and at its distal end two bristles, one simple, one plumose ; the last joint terminates abruptly; at its distal end is a dorso-ventral row of four bristles, the second ventral one plumose, and rather stronger than the rest, which are simple. In *Balanus* there are at this stage no dorsal bristles or hairs, but in *Lepas* there is a bunch of hairs at the distal end of the third joint.

The *antennæ* are large and consist of a two-jointed *protopodite*, an *endopodite*, and an *exopodite*.

The protopodite is two-jointed and very stout. Its proximal joint is attached to the body, not far from the anterior corners of the mouth, by a rather narrow neck, which soon expands into an almost spherical distal portion; this is produced on its

ventral and inner side into a very strong conical process or gnathobase (*gn.*), with two sharp teeth at its apex, surrounded with small spines and hairs. This process works with the limb in a horizontal circle towards or away from the mouth, close behind which the teeth are situated when the appendages are directed backwards ; it has also an independent motion of its own, being articulated with the globular head of the joint, and moved, as I have been able to observe, backwards and forwards by means of a powerful muscle (fig. 142, *gn.m.*), for the reception of which the joint is swollen. The second joint is also rounded but much smaller than the first, and gives rise on its inner and ventral side to a powerful hairy spine, with a strong conical base, which is furnished with two short bristles, one simple, the other plumose. The dorsal edge of both joints has in *Lepas* a line of short hairs, scarcely represented in *Balanus*.

The endopodite (in accordance, perhaps, with the function of the spines of its base) is not sharply separated off from the protopodite, the basal joint not being distinctly articulated. It consists of three joints ; the basal one is rounded, and has on a prominence on its inner and ventral side two long spines with numerous hairs arranged regularly down both sides. The second joint is smaller and cylindrical, and has two ventral stiff plumose bristles placed side by side at its distal end. The third joint is oval, and provided with three bristles, the most ventrally situated simple, and the two others plumose.

The exopodite is longer than the endopodite, and lies dorsally to it. It consists of nine joints, of which the first seven are cylindrical, and diminish regularly in diameter from the proximal extremity ; the eighth is conical, and the ninth minute. The dorsal side of the whole exopodite is marked by a band of short hairs continuous with those on the protopodite. The first joint has no other appendages ; the second and third have a ventral row of hairs ; the third has, in addition, a simple bristle at its distal end ; the next four joints (4 to 7) have each at their distal end a long bristle, plumose on both sides. The bristle of the eighth is very strong and almost as wide at its base as the joint itself, and is plumose on both sides in *Balanus*, and more strongly so on one side in *Lepas ;* the bristle of the ninth is smaller and simple. The axis of the exopodite is generally curved somewhat dorsally.

. If the term mandible were used in a descriptive sense, the second pair of appendages of the Cirripede Nauplius should certainly be so termed, but since the third pair probably morphologically represents the first and strongest pair of jaws in the adult, the name may be reserved for the latter.

. The *mandibles*, or third pair of appendages, are smaller than the antennæ, and consist, likewise, of a protopodite, endopodite, and exopodite.

The protopodite is two-jointed. The basal joint is attached by a rather narrow neck close behind the antennæ, and behind and outside the mouth ; it enlarges distally to form a rounded head which gives off on its ventral and inner side a strong spine, plumose on two sides, and which works horizontally (with the limb only)

behind the mouth, and below the bidentate process of the antennæ. The second joint is large, flattened from side to side, and expanded distally ; on its lower side is a semicircle of hairs (double in *Lepas*) with the concavity turned inwards ; this joint also has two spines, which are bent inwards, and plumose on two sides, the proximal one being stouter, especially in *Balanus ;* in *Lepas* both rather resemble stiff bent plumose bristles.

The endopodite is two-jointed ; and, as in the case of the antennæ, not so distinctly articulated as the somewhat shorter and more dorsally situated exopodite, and not sharply marked off from the protopodite. The first joint is short and expanded ventrally ; it bears on its lower side a semicircle of hairs in *Lepas*, in addition to which is a distal group of hairs ; the prominence bears three plumose spines bent inwards, one of which, situated most dorsally, is stouter than the other two, especially in *Balanus*. The distal joint is bluntly conical, with a ventral notch not far from the end ; this gives insertion to two stiff bent bristles placed side by side, one on the outer, and one on the inner side, the former plumose, and the latter simple ; at the base of this joint is a small semicircle of hairs on the outer side in *Lepas ;* at the end of the joint are three stiff bristles, two more ventrally situated, placed side by side, and one terminal ; all three are simple.

The exopodite is five-jointed and longer than the endopodite. The joints taper off distally, each bearing a bristle ; that on the first joint is simple and shorter than the rest ; the next three are plumose on both sides, and the fifth is the direct .continuation of the small spindle-shaped fifth joint, and has long secondary hairs on the ventral side only.   The dorsal side of the exopodite has also a row of simple hairs.

An axial portion can be distinguished within each hair or process, which has been regarded as a nerve ; it is, however, nothing more than the hair of the next stage, as can readily be seen just before a moult.

TABLE showing Number of Joints and Processes in the Appendages of the Cirripede Nauplius of Stages 1 and 2.
(*t* = terminal; *l* = lateral.)

Antennules.

	Stage 1.	Stage 2.
Joint 1 . .	..	..
„ 2 . .	1 bristle (*t*)	1 bristle (*t*)
	2 bristles (*t*)	2 bristles (*t*)
„ 3 .	1 bristle (*l*)	1 bristle (*l*)
„ 4 .	4 bristles (*t*)	4 bristles (*t*)
„ 4 .	..	..
Total .	**8**	**8**

Antennæ.

	Stage 1.	Stage 2.
Protopodite joint 1 .	1 process	1 process
„ 2	1 spine .	1 spine .
Endopodite „ 1	1 bristle.	2 bristles
„ 2	2 bristles	2 „
„ 3	2 „	2 „
Exopodite „ 1	3 „	3 „
„ 2	..	..
„ 3	1 bristle.	1 bristle.
„ 4	1 „	1 „
„ 5	1 „	1 „
„ 6	1 „	1 „
„ 7	1 „	1 „
„ 8	0	1 „
„ 9	0	1 „
Total .	**15**	**18**

Mandibles.

	Stage 1.	Stage 2.
Protopodite joint 1 .	1 process	1 process
„ 2	1 spine .	1 spine .
	1 bristle .	1 bristle
Endopodite „ 1	1 spine .	1 spine
	1 bristle .	1 bristle
„ 2	2 bristles (*l*)	2 bristles (*l*)
	3 „ (*t*)	3 „ (*t*)
Exopodite „ 1	1 bristle .	1 bristle
„ 2	1 „	1 „
„ 3	1 „	1 „
„ 4	1 „	1 „
„ 5	0	1 „
Total .	**14**	**15**

(H.) *The Alimentary Canal.*

. *The alimentary canal* already possesses the three divisions characteristic of that of the adult. These have been noted by Münter and Buchholz. They are the *œsophagus (stomodæum)*, the *stomach* (mainly *mesenteron*), and the *intestine (proctodæum)*. It is not possible, I think, to distinguish, as these authors have done, a posterior section or rectum.

The *œsophagus (œs.)* commences with a mouth provided with a thickened chitinous margin, and runs forward, as Darwin has observed, from immediately behind the base of the labrum, as a bent tube, the course of which is indicated in the figures 145 and 158. The first part is horizontal, and leads close to the lower sloping surface of the head, which may be described either as the base of the labrum, or as a part of the head continuous with it. It then bends round in a gentle curve, and passing between the circum-œsophageal connectives into a short vertical section placed immediately behind the brain, which can be nearly always seen in optical section in both dorsal and ventral views (fig. 157). Its termination projects (as in the adult) backwards into the stomach (figs. 136*c* and 139*a*). The tube is slender and consists of a single layer of polyhedral cells (figs. 136*c*–137*d*), each with a relatively large nucleus, often containing a single nucleolus; about four to eight of these cells are seen in transverse section; the lumen is distinct, and circular or quadrangular in section. When living the œsophagus is seen to be transversely striated, and to be undergoing peristaltic contractions (which have led to its being regarded as a heart). The appearance and movement are due to the presence of a single layer of circular muscles, which are readily seen when the motion ceases, or in stained specimens. They consist of a number of simple spindle-shaped fibres, apparently unstriated at this stage, short and broad when contracted, and much elongated at other times. The œsophagus is attached to the walls of the labrum by a number of contractile fibres (figs. 145 and 158) which radiate out from, and serve to dilate it.

The *stomach (st.)* consists of a large simple globular or spheroidal sac, usually of a green or brownish colour, and occupying a considerable part of the anterior of the body of the Nauplius.

It has a distinct enteric cavity which arose for the first time just before the hatching of the Nauplius. It has now also an ordinary cellular structure, and as may readily be determined the cells arise by direct transformation of the yolk-endoderm pyramids. Just before hatching it will be remembered that the whole stomach consisted of a number of yolk-endoderm cells, each consisting mainly of yolk granules but containing a single nucleus. Each pyramid has now become directly transformed into an endoderm cell (fig. 141) by conversion of the yolk granules into protoplasm. The endoderm cells at first large (fig. 141), soon become much smaller by rapid division (figs. 156, 136*a*–*d*), and all traces of the yolk disappear. The endoderm is thus not, as has been supposed by Hoek, a new formation independent of the yolk segments. My account agrees rather with that of Nasonov, who describes the

nuclei in the endoderm segments as becoming peripheral, the granules diminishing in number, and the endoderm segments becoming smaller as a cavity arises between them.

The wall of the stomach consists of a single layer of cells of two sorts : the first are polyhedral cells larger than those of the œsophagus and intestine, and containing a large round nucleus with a nucleolus (figs. 136a-d); they occupy the whole wall of the stomach, except a small posterior section, and are directly derived from the yolk-endoderm cells. In the neighbourhood of the opening into the intestine the cells forming the posterior wall of the stomach (st.gl.c.) are much narrower and higher, forming a columnar epithelium, the nuclei of which are oval and arranged radially ; the protoplasm of these cells is distinctly radially striated and stains much more deeply than the rest of the cells of the stomach (figs. 136a, 136b); these cells are part of the proctodæum. The opening of the pyloric end of the stomach is small (fig. 136a) and situated dorsally, owing, apparently, to a downward growth of the hinder wall of the stomach. The ventral side of the pyloric end projects below the anterior end of the intestine (figs. 136a, 136b). The statement of BUCHHOLZ that the stomach is furnished with circular muscles requires limitation, as they occur only on the portion at the posterior end. These muscles are quite similar to those of the œsophagus and intestine ; circular muscles are thus apparently limited to the part of the gut arising from the stomodæum and proctodæum. Extending from the posterior end of the ventral prolongation of the pyloric end of the stomach to the middle of the intestine is a group of simple unstriated longitudinal muscular cells (figs. 136a, 136b, 142, 146).

The *intestine* (*int.*) varies in appearance from a narrow, tubular, distinctly trans-versely-ringed organ to a broadish oval sac, according to the state of contraction of the muscles surrounding it. It consists, like the œsophagus, of an epithelial and muscular layer. The former is a simple layer of low, rounded or flattened, cells (figs. 136a, 136b), not always distinctly marked off from one another; the nuclei are small and contain a single nucleolus. The muscles are simple, unstriated, spindle-shaped fibres with a single nucleus, varying much in appearance according to the state of contraction. The anus, as already stated, is a narrow aperture, placed between the tail and caudal spine.

In addition to these epithelial and muscular elements, the walls of the alimentary canal are completed by mesodermic cells, which will be referred to again.

The alimentary canal is attached between its extremities to the body walls by fibres, some of which are probably contractile ; just before the anus is a transverse sheet of tissue, attached to the body walls all round the anus (figs. 141, 156, *an.dil.*), and probably including many contractile fibres.

(J.) *The Nervous System.*

The *nervous system* at this stage differs somewhat in the Lepads (*Lepas anatifera,*

L. pectinata, Conchoderma virgata, Dichelaspis Darwinii) and Balanids (*Balanus perforatus, Chthamalus stellatus*), but the structure is very uniform in the members of the former family.

None of the existing descriptions of the nervous system are complete; most observers have seen the brain alone, and have described and figured even that incorrectly. KROHN and NUSSBAUM alone appear to have seen the circum-œsophageal ring and sub-œsophageal ganglion, the former in a Balanid Nauplius, and the latter in *Pollicipes;* but the figures of both are diagrammatic, and scarcely any details are given.

In order to make out the nervous system satisfactorily, it is necessary to stain and clarify the more transparent Nauplii, and to cut sections of the more opaque.

In the Lepads (figs. 154, 156–159) it consists of a small brain of two lobes (*br.*), closely applied in the middle line, and staining more deeply than the circum-œsophageal connectives arising from them, owing to the more numerous ganglion cells present. Each lobe is spherical, and situated close to the ventral surface; it passes backwards into one of the two connectives (*c.o.c.*).   The latter have ganglion cells all along their course, but present in much smaller numbers than in the ganglia.   The connectives diverge and form an elongated loop on each side of the œsophagus and terminate immediately behind the mouth in two triangular* ganglia (figs. 159, 160), closely applied in the middle line to form the sub-œsophageal ganglion (*sub-œs.g.*), the posterior wings of which project (in the direction of connectives to be formed later) close beneath the flexor arcs about as far back as the level of the posterior end of the stomach, but not so far as the end of the labrum ; the sub-œsophageal ganglion is thus concealed both in dorsal and ventral views.

Each lobe of the brain is excavated by the spherical base of the frontal filaments, and supports on its dorsal surface close to the middle line one-half of the Nauplius eye.

All parts of the nervous system at this period are in close connection with, or rather form thickenings of, the ectoderm.

In *Chthamalus* (fig. 149) the nervous system resembles that of Lepads, with the exception of the addition of the pair of accessory lobes (*br.acc.l.*) described in *Balanus*.

In *Balanus perforatus* (figs. 137c to 139a, 141, 143, 145) the nervous system is more complex; it here consists of a complex brain, circum-œsophageal connectives, and a sub-œsophageal ganglion, all in the closest relation with the ectoderm.

The brain consists of a number of lobes, of which two—the *anterior lobes* (*br.*)— resemble and correspond to the simple lobes of Lepads.   These are more or less reniform, and closely applied to one another anteriorly, but separated behind by two other lobes.   They are excavated anteriorly by the bases of the frontal filaments, and support close to the middle line the pigmented plates of the Nauplius eye ; the

* The shape was not made out in *Dichelaspis.*

lateral portions, not as yet distinct from the rest, will form the compound eyes, and may be termed the *optic tracts*. The anterior lobes are thus sensory in function.

Between the hinder ends of these lobes, and distinct from them, are two smaller hemispherical lobes (figs. 137*d*, 141, 143, *br.acc.l.*), closely applied to one another in the middle line, and which may be termed the *accessory lobes*. I have failed to recognize their significance.

The whole of the anterior lobes consist of small ganglion cells, the nuclei of which are closely crowded and show a number of deeply staining granules. The same may be said of the accessory lobes; the latter, however, rest upon a large spherical mass of grey matter (figs. 137*a* to 137*c*, *br.c.l.*), also associated anteriorly with the anterior lobes, and posteriorly almost in connection with the grey matter of the connectives.

On the ventral side of this *central lobe* is a second accumulation of ganglionic matter on each side (fig. 137*a*, *br.p.l.*), composed mainly of ganglion cells and forming the posterior section of the ventral portion of the brain; these may be termed the *posterior lobes*. They meet in the middle line and give off posteriorly the two circum-œsophageal connectives (figs. 143, 145, 137*a*, *c.o.c.*). The circum-œsophageal connectives separate and pass in an oval loop round the œsophagus; they consist mainly of grey matter, but have a group of ganglion cells all along the ventral and outer border (figs. 136*a*, 137*b*).

The connectives give off on their ventral side shortly after leaving the brain two ventral lobes—the *nerves to the labrum* (*lbr.n.*)—which pass into (figs. 136*c*, 136*d*) and occupy the dorsal section of the sides of the proximal lobe of the labrum; they run nearly to the end of this lobe (fig. 145). These branches consist mainly of ganglion cells; their origin and fate are closely connected with those of structures apparently of glandular nature, more fully developed at a later date.

The connectives have in front few ganglion cells, but these soon increase in number, and limit the grey matter to their inner and dorsal side (figs. 137*b*, 137*c*); the connectives unite behind the mouth and form a broad and thick plate of ganglion cells, bilobed and still containing grey matter anteriorly (fig. 137*b*), but simple posteriorly (figs. 137*c*, 137*d*); the posterior section finally becomes thinner and disappears in the setose area.

The antennules arise precisely at the level of the posterior lobes, and are in close connection with them, a short prominence of the lobe (fig. 137*a*) sometimes projecting into the cavity of the appendage.

The antennæ arise at the level of the circum-œsophageal connectives (fig. 137*a*), and the ganglion cells on their outer and lower border sometimes project slightly into the cavity of the appendage.

The mandibles arise just opposite the anterior end of the large sub-œsophageal ganglion with which they are in close relation (fig. 137*d*).

(K.) *The Sense Organs.*

Of sense organs two kinds only can be detected at this stage. These are the Nauplius eye and the frontal filaments.

The *eye* (*Npl.eye*) consists in all the species of two oblong black pigment plates generally placed together (but sometimes separated by a small interval) in the middle line, like the two halves of an open book, each on the dorsal side of one of the anterior lobes of the brain, and not separated from the latter by an interval as figured by Nussbaum. Sometimes, especially when the eye is injured, a reddish colouring material may also be seen. I could detect no special cells in connection with these plates; the ganglion cells below are apparently similar to those elsewhere.* The lens stated by a number of observers to exist, but denied in *Balanus balanoides* by Hoek, is equally absent in all the species I examined.

The (*fr.fil.*) are two delicate transparent filiform organs inserted, as Fritz Müller (16) and Hoek (30) have supposed, directly on the brain.

They consist of a cuticle,† separated from that of the rest of the body by flexible membrane, and of an axial strand. The sheath or cuticular portion consists of two joints, and is never multi-articulate as figured by Spence Bate; the basal joint is short and conical, and the distal filiform and closed at the end. They are directed downwards and a little forwards, the distal joint having a ventral flexure. The basal portion arises on the ventral surface of the body at the level of the eye, and is situated on the ventral surface of the anterior lobe of the brain, inside which its continuation expands in the form of a spherical vesicle seen on each side of the eye (figs. 137*b*, 137*c*, 141, 142, 145, 149). In most cases the contents of the sheath are not distinctly seen, but in favourable sections a finely or coarsely granular portion is seen occupying the axis of the filament, and expanding within the spherical base. This axial portion much resembles in appearance the grey matter of the brain, and is probably of a nervous nature and in communication with the brain at the base of the sac, though I was not able to observe this connection.

There are no muscles passing to the frontal filaments, which can therefore be moved only in a passive manner.

(L.) *The Muscles.*

The muscles proper to the wall of the alimentary canal have already been described. There are others which are equally unstriated and which cause dilatation of different parts of the gut. These are most prominent in connection with the œsophagus, which they connect with the wall of the labrum (figs. 145). They are also found in connection with the hind-gut, and nucleated fibres, probably contractile, are found in the transverse membrane close to the anus, which they serve to dilate.

* Special cells in connection with the pigment plates are seen in some of my best preparations of later Nauplius stages.

† The cuticle, like that of the rest of the body, is insoluble in hot strong caustic potash.

The disposition of the striated muscles has been described in most detail by MÜNTER and BUCHHOLZ, and by HOEK, and the accounts of these authors agree in most points with my own.   The muscles, however, stated to go to the fronto-lateral horns, do not exist, those of the antennules, or the glands of the horns, having been apparently mistaken for such.   No muscles pass to the labrum, and any movements that organ undergoes must be brought about accidentally.   It seems probable, however, that the distal lobe can move on the proximal, since two strong fibres (figs. 142, 145) (possibly unstriated muscles) pass from the dorsal sides of the proximal lobe of the labrum to the base of the distal in *Balanus*.   As stated by the above mentioned observers, muscles pass from the median dorsal line of the carapace to the limbs (*app.d.m.*).   They radiate out from the central area of the carapace as six systems, one passing through the dorsal section of the body cavity to each limb.   The muscles to each limb do not all arise close together; thus, for instance, some muscles going to the mandibles arise in front of some going to the antennæ.   There is on the ventral side an almost equally strong system of muscles (figs. 137*b*, 137*c*, 154, *app.v.m.*) which have been overlooked owing to their being hidden by the stomach in dorsal views and by the labrum in ventral views.   They are readily seen when ventral views are obtained of Nauplii in which the labrum has been bent forward, and in lateral views, or even in dorsal views, by focussing deep down in transparent specimens.   The figures will sufficiently indicate the disposition of these twelve groups.

The powerful flexor muscles (*fl.ta.*) (in ventral and lateral views) of the tail pass from the carapace close to the attachment of the secreting end of the fronto-lateral glands downwards and backwards to the base of the tail.   They have been already correctly described by BUCHHOLZ and by HOEK.   The muscles to the limbs are all transversely striated (fig. 138*a*).   They are inserted directly on the cuticle at both ends.   The histology of the several species I observed much resembles that described by HOEK in *Balanus balanoides*.   The transverse striations are clear, and each fibre has one or more, commonly two, protoplasmic corpuscles on one side.

Of a number of muscles limited to the limbs and causing movement of one part on another, one only needs special mention.   This occurs in the second joint of the pro-topodite of the antennæ, and is a short powerful fan-shaped muscle causing motion of the strong gnathobase of this appendage (fig. 142).

(M.) *Other Mesodermal Elements.*

The body cavity is bounded on one hand mainly by the ectoderm of the body walls and appendages, and on the other by the walls of the gut.   At intervals more or less flattened mesodermic cells are scattered along these walls as well as on the brain, gland-walls, and muscles (figs. 149, 156).   Others stretch across the cavity from one wall to the other, or from one organ to another (figs. 149, 151, 156); these occur throughout the body, but are especially distinct in the anterior part; here bi-, tri-,

or multi-polar cells lie suspended by fibres between the structures mentioned. Though often single, mesodermal cells are very commonly distributed in groups ; one group is generally found in the angle between the fronto-lateral glands and the front wall (fig. 156). Four connective tissue bands are very conspicuous and constant in position in *Lepas* and *Conchoderma;* they run from the brain to the dorsal surface of the carapace near the front margin, as shown in all the dorsal views of these species ; the two outer are specially prominent, and contain three or four cells about the middle of their length ; they branch at their ends into a star-like series of fibres (*st.b.*). Two more of these stellate bodies are also found near the posterior end of the line bounding the lateral zone of the carapace.

In certain regions there is a peculiar tissue (*ves.t.*) which is difficult to understand ; this consists of an excessively delicate, vesicular, transparent, and colourless tissue, prominent at the sides of the intestine and stomach (figs. 141, 156), and occurring also in the caudal spine, tail, and in front of the fronto-lateral glands. It consists of very thin walls, cubical, or polyhedral vesicles, only staining slightly with reagents. In the tail and caudal spine a small quantity of granular staining matter, with one or two nuclei, can be detected in association with it ; elsewhere the tissue is not sufficiently distinct from the neighbouring tissues for examination ; it is probably to be regarded as a vesicular connective tissue.

The nuclei of the mesoderm cells are generally fairly large, the cells themselves not present in great numbers, and their boundaries tolerably distinct.

In the labrum there are, in *Balanus*, a number of cells (figs. 142, 145), into close connection with which the nerve of the labrum comes ; the nuclei of these appear very similar to those of the nervous system, but generally stain a little less deeply ; they occur all along the sides of the proximal lobe, and form a band extending transversely across its distal end.

In the Lepads (*Lepas, Conchoderma, Dichelaspis*) they form a small accumulation of cells (figs. 157, 159), (*lbr.c.*) embracing the diverging arms of the axial gland.

Of cellular elements floating about in the plasma at this stage I have no observations, though they occur sparingly in later stages.

Oil globules are not unfrequently seen embedded in the outer wall of the stomach.

(N.) The Ectodermal Thickening of the Tail, or " Ventral Plate."

The ectodermal thickening of the tail (*ta.th.*) has been passed over in silence by most authors. HOEK (30) alone has seen and figured it (in *Balanus balanoides*), but gave no interpretation of it. GROBBEN (35) has given a different figure, and states that HOEK's description is inexact ; he describes and figures a small band of mesoderm cells placed on each side below an ectodermal thickening, and consisting in the Nauplius examined of three cells, the most posterior of which was largest. He supposes from this that the mesoderm of *Balanus* arises from two pole cells. Now it follows from the foregoing description of the origin of the mesoblast at an early stage

that this is not so, and I believe, moreover, that GROBBEN's figure is much less correct than the one of HOEK he criticises.* I tried the method recommended by GROBBEN for small Entomostraca (BEALE's carmine), but found the maceration too great in the case of Cirripede Nauplii, while specimens either unstained or lightly stained with borax carmine gave much better results.

The ectodermal thickening in *Balanus perforatus,* at Stage 1, is shown in fig. 146, and at a later period (Stage 2) in fig. 147. It consists at first of two plates of considerably-enlarged ectoderm cells which extend in a single layer from shortly behind the flexor muscles to the end of the tail. They are distinct in front, being separated clearly by a median groove, but unite together as the tail narrows behind. In Stage 1, the cells are relatively larger and fewer, they possess very distinct, clear, and large nuclei, each generally with a large nucleolus, and are arranged more or less in longitudinal (about four visible ventrally) and transverse rows. The anterior margin is nearly transverse. Well on in Stage 2, the cells are much more numerous and smaller, but still show an arrangement in longitudinal rows. In both stages the thickening extends obliquely upwards and forwards, but does not pass above the level of the intestine, so that the bands are truly ventral. The arrangement of the cells in longitudinal rows appears to indicate transverse division of a primitively smaller number of cells arranged transversely.

In *Lepas* and *Conchoderma* the thickening is of much less extent. Over the greater part of the tail the ectoderm has its usual characters, but in the anterior region just beneath, and closely following the extra-maxillary bands, are two oblique rows of thickened ectodermic cells forming the rudiments of the ectoderm and mesoderm probably of the fourth pair of appendages alone (figs. 160, 163, &c.).

In *Dichelaspis* and *Chthamalus* the thickening is similarly represented by a minute group of cells.

The appearance of these bands in *Balanus* strongly reminds one of the mesoblastic bands, so well-known to embryologists in other types. They lie, however, as a simple layer of cells (figs. 136*b*, 139*c*) immediately beneath the cuticle, and pass in front into ordinary ectoderm cells (fig. 139*b*). They are rather to be compared with the greatly-enlarged and symmetrically-disposed cells described by DELAGE in the same region in *Sacculina,* and seen by myself in *Peltogaster.* The fate of these cells will be traced in the second part of this work.

(O.) *The Body Cavity.*

The body cavity at this stage is a space (*b.c.*), devoid of a special epithelial lining and filled with a plasma containing few or no corpuscles. This is situated between the body wall and the alimentary canal, and extends into the labrum, appendages, tail, and caudal spine.

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* It seems to me probable that the Nauplius examined by GROBBEN was that of *Chthamalus stellatus* and not a *Balanus* at all, in which case the difference between his and HOEK's description is more easily understood.

PART IV.—PHYSIOLOGY.

The physiology of the Nauplii is practically the same for all stages, and the following remarks apply equally to the earlier or later stages.

(A.) MOTION.

The movements of the Nauplius are brought about exclusively by the activity of the three pairs of appendages. The three pairs are all moved together in a horizontal direction; the bristles and hairs on the appendages are vertically arranged so as to give the greatest effect to this stroke. The Nauplius thus moves by a series of jerks which follow one another with great rapidity in the newly-hatched Nauplii, but more slowly afterwards, especially in the loosely-built Nauplii of the Lepads, where, in Stage 2, the movement is comparatively slow. In *Balanus perforatus,* the Nauplius, after the first moult, moves at the rate of about one millimetre per second, a rate equal to rather more than one hundred times it own length per minute, the strokes being at the rate of several per second.

The tail and caudal spine act as a rudder; when in a line with the body they serve to steady and straighten the course, which is often very direct. When the motion is to be suddenly arrested and reversed, the tail and caudal spine are sharply bent down at an angle by means of the flexor muscle, and the Nauplius turns a somersault.

(B.) NUTRITION.

The small and chitinized mouth is situated, as has been seen, at the base of the labrum.

I have no direct observations on the nature of the food taken in the natural state, nor do sections of the stomach show anything recognizable.

The fact that the stomach is often green has led to the statement that the Nauplii feed on plants; it appears to me possible that the green colour is simply due to the alteration in colour of the original yolk.

The small size of the mouth indicates that only small bodies or substances in solution can be taken up. The presence of powerful jaws on the appendages indicates that bodies of a resistent nature have sometimes to be masticated. The abundance of stiff spines and bristles on the inner side of the endopodites, their position at some distance behind the mouth where they would not be of much use for mastication, and the curvature towards the space behind the mouth, seem to suggest the function of holding bodies of some size. DARWIN has already expressed his belief that these spines are adapted for grasping rather than masticating. The rich plumose character of the hairs on the inner sides of the appendages, and the direction of the hairs on the

setose region and on the labrum, appear to indicate a function of retaining small particles within the sphere of attraction of the œsophagus. The œsophagus, as previously mentioned, undergoes continuous contraction and dilatation by means of the muscles with which it is furnished, and constitutes a suction-pump which causes an intermittent stream of water to enter the mouth.

It seems probable that the mode of nutrition is as follows :—The powerful strokes of the three pairs of appendages sweep backwards and inwards any small organisms or particles entangled between the network of hairs and bristles ; the œsophageal pump must cause a current towards the mouth, and the anal and other lateral rows of hairs will prevent the bodies from passing dorsally ; the presence of the forwardly-directed hairs of most of the setose region will hinder their escape backwards. Any substances in solution or small particles in suspension will be thus drawn into the mouth, while the larger bodies or organisms held fast by the spines of the endopodites, and possibly paralyzed by the secretion of the axial gland of the labrum, will be torn up and masticated by the powerful movable gnathobases of the antennæ, and the fragments, retained by the hairs of the labrum and setose region, sucked into the mouth.

The Nauplii are apparently predaceous, and I imagine their food consists, in addition to the more minute microscopic organisms, of small soft-bodied or tolerably resistent animals, such as would occur in the pelagic waters inhabited by the Nauplii.

That minute organic particles and substances in solution and suspension are taken into the body I have experimental evidence to show.

Though the Nauplii of *Balanus*, *Chthamalus*, and *Lepas* refused to eat starch, they greedily took up water containing carmine, indigo-carmine, methyl-blue, litmus, &c. In the case of the first three substances considerable accumulations were formed after a short time in the stomach and intestine, but the greater part of the carmine was passed out unabsorbed.

Litmus solution either remained blue or turned faint red in the stomach and hind gut, this showing the presence at times of an acid digestive juice ; the secretion of this probably takes place from the deeply-staining striated columnar cells forming the hind wall of the stomach.

The fæcal matter is expelled by the contractions of the pyloric portions of the stomach and of the intestine.

As in other Nauplii there were no special organs of circulation, the movements of the œsophagus and gut serving, no doubt, to fulfil this function, as well as that of respiration.

(C.) SECRETION.

In addition to the glandular epithelium of the stomach the only important secreting cells are the unicellular glands opening on the carapace and labrum, viz., the fronto-lateral gland, the lateral gland, and the axial gland of the labrum. It is only after the first moult that the fronto-lateral, and probably the other glands open

to the exterior, though they are fully developed and contain abundance of the secreted globules while the Nauplius is still within the egg membrane. The secretion of the fronto-lateral and lateral glands consists of transparent globules provided with a resistent pellicle, and which, though closely pressed against one another into polyhedral bodies within the cavity of the gland do not fuse ; they are not dissolved by water, alcohol, weak acids or alkalies; they show no acid or alkaline reaction, and take up no colouring matter. The membrane of the gland itself and of the globules is possibly of a chitinous nature, since the sacs containing the glands, and presumably secreted by the glands themselves at an early stage, consist of a refractive membrane, while the lumen of the fronto-lateral horns is often partially interrupted by septa of apparently similar nature and formed by the adhesion of the membrane of the globules to the walls of the horns (see p. 174). The chitin, however, must be of a delicate nature, for while resembling the cuticular covering of the whole body in being soluble in warm acid (HNO_3), it also dissolves in hot caustic potash, which the ordinary cuticle resists.

It is worthy of note that the period of secretion of all three glands is almost coincident with that of the free life of the larva, the secretion passing to the exterior after the first moult, $i.e.$, shortly after hatching, and ceasing when the Cypris-stage becomes fixed, but occasionally while free. It is possible that no new secretion is formed during the Cypris stage, and that the glandular material present is simply a relic of that produced during the earlier stages. The period of secretion would then be that during which the larva feeds, for, as is well known, the Cypris form does not feed.

The presence of sharp points at the ends of the fronto-lateral horns in the earlier stages, and of a strong spine projecting from the horn during the later stages seems, as CLAUS and HOEK have already pointed out, to indicate that they may be piercing organs provided with poison glands. It is to be observed that the area covered by the horns is that included by the sweep of the appendages, and that any organism paralyzed by the secretion would tend to be swept towards the region of the mouth.

The lateral gland and the other glands of the carapace of later stages appear from their position more adapted for protection than for securing prey.

The position of the gland on the labrum and the presence of a perforated tooth for the passage of its secretion, taken in conjunction with the supposed free motion of the distal lobe of the labrum, indicate that it may be used to pierce and paralyze organisms held by the stiff spines on the endopodites of the antennæ and mandibles in the way supposed in the preceding section.

(D.) EXCRETION.

With the object of determining whether any portions of the body were specialized for secretion, the Nauplii of *Balanus perforatus* were fed with powdered carmine,

indigo-carmine, Bismark brown, and methyl-blue in sea-water. All these substances were readily taken up, but, though the carmine and indigo-carmine formed large accumulations in the stomach and intestine, none appeared elsewhere; nor is it probable that the alimentary canal has an excretory function in the way found by CLAUS for Copepods (58), as colouring matter did not occur in any of the cells of the digestive tube. The Bismark brown on the other hand appeared to be digested and excreted again all over the body, so that ectoderm, muscles, and nerve cells, etc., appear to have a certain excretory power. With methyl-blue more definite results were obtained : the colouring matter is excreted in the ectoderm as small blue granules which give a blue tint to the whole dorsal surface, the carapace especially, of the Nauplius ; the excreted granules also extend into the base of the first and second pair of appendages, and are especially numerous in the epithelium of the labrum ; they are also abundant in the mesodermal cells covering the stomach and scattered about on the muscles. They generally occur in circular patches (figs. 148, 148a), corresponding with the protoplasmic accumulations. In order to make certain that the particles were not taken up by the ectoderm, Nauplii which had fed on the colouring matter, and had a considerable accumulation in the stomach, but none in the ectoderm, were isolated and placed in fresh sea-water; after a lapse of some time the methyl-blue was seen to be excreted precisely as before.

It would thus appear that the lining of the body cavity has a special excretory function, more particularly in certain regions.

The Nauplii of *Lepas anatifera* and *Chthamalus stellatus* excreted methyl-blue in the same manner.

No excreted particles were found in the fronto-lateral or lateral glands, or in the axial glands of the labrum.

(E.) PERCEPTION.

The frontal filaments from their intimate connection with the brain are no doubt sensory organs. Their position and ventral direction would render them well adapted to test the chemical nature of the substances with which they came into contact before reaching the mouth, and the term " olfactory filaments " applied to them first by FRITZ MÜLLER is, no doubt, suitable.

The perception of light by the Nauplii of most Cirripedes examined is very marked, but whether this takes place over the general surface as in some animals (78), or is limited to the Nauplius eye or some other spot there is no evidence to show.

(F.) EFFECT OF STIMULI.

The reaction to light is usually very marked and has been already treated of at length by Dr. LOEB and myself. It will be sufficient to state here that during motion the Nauplii of *Balanus perforatus* commonly tend to place their longer axis parallel

to the rays of light; that light of sufficient intensity and duration ordinarily causes them to turn the oral pole away from the light, while weaker light has after a time the contrary effect. For fuller details as to the action of the rays of different refrangibility, and of sudden increments or diminutions in the intensity of light, I must refer the reader to the original communication. We have there shown that the effect of this must be to produce the daily, and possibly also the annual, migrations to and from the surface, such as have been found to take place in the Nauplii of *Lepas fascicularis* (28) and many other pelagic forms.

The wide prevalence of this effect of light on animals (and even on plants), as shown by the movements of such pelagic forms (whether fresh water or marine), hemi-pelagic forms, and larvæ which are not pelagic forms, which are so different that we must suppose the effect to have arisen independently in the different groups, appears to indicate the great physiological importance of the periodic movement. Whether this is to regulate the amount of intensity of light received, or has some other object is not clear.

The immediate effect of an increase of heat above the normal winter temperature appeared to be at first a great increase in the activity of movement, but after a certain temperature was reached the Nauplii became less active, and if the temperature was not then lowered died; if the heat was again diminished an increase of activity took place. The maximum, optimum, and minimum temperatures for the activity of the Nauplii were not determined.

(G.) Effect of Gravity.

Gravity has apparently a definite effect on the Nauplii of *Balanus perforatus* (and possibly on those of other species, but these were not examined in this respect). If they are very closely examined with a lens or low power of the microscope it is seen that the Nauplii generally move along with their ventral surface upwards; this is specially clear in the case of the larger and older Nauplii, but in consequence of their small size and rapid motion is difficult to make certain of in the younger. The circumstance has apparently no relation with the surface of the water or with the bottom of the vessel, as it occurs in whatever part of the vessel the larvæ are.

PART V.—GENERAL CONSIDERATIONS.

(A.) Segmentation of the Ovum.

The segmentation of the ovum has been described by the best observers as total, followed by epiboly. The migration of the protoplasm to one pole does not, however, as has been generally supposed, represent simply a division into epiblast and hypoblast; since the supposed representative of the latter (the yolk) is at first devoid of a special nucleus, and furnishes, moreover, at a slightly later period material

for the production of new epiblast cells. The process is probably simply the formation of a telolecithal egg.

Fig. 3.
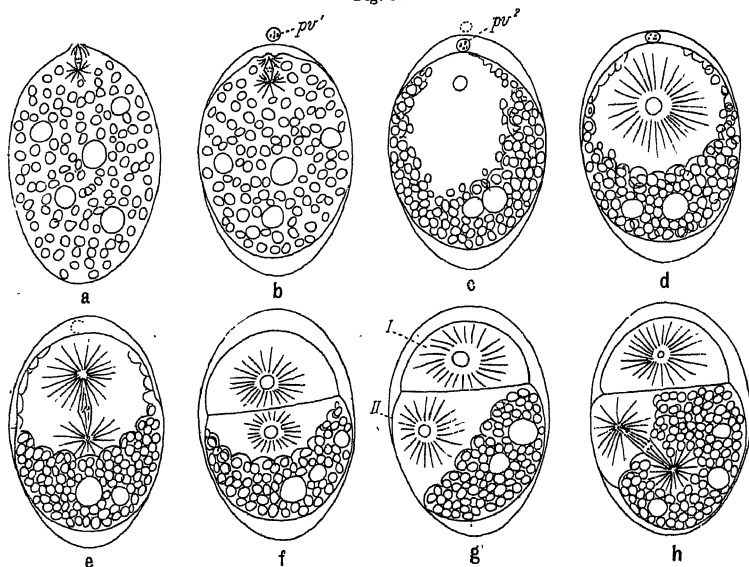
a...s. Diagrams illustrating the mode of segmentation of the Cirripede ovum, the formation of the blastoderm, and of the three germinal layers. The protoplasm is left white; the yolk is granular, with oil-drops. I, II, and III, the three first blastomeres, formed directly from the yolk; $pv.^1$, first polar body; $pv.^2$, second ditto. (The stellate figures at the ends of the directive spindles in figs. a and b were inserted through inadvertence.)

In a, the formation of the first polar body is shown; in b, that of the second, from the contractile ovum now withdrawn from the newly-formed vitelline membrane; in c, the protoplasm is collecting at one end, and the segmentation-nucleus is becoming conspicuous; in d, the segmentation-nucleus is preparing to divide; in e, the protoplasm is collected at the anterior end, and the nucleus is dividing; in f, the first blastomere has been cut off from the yolk; in g (lateral view), the second blastomere is forming; in h, the nucleus is dividing to form a third blastomere; in j' (side view) and j'' (front view), the second blastomere is cut off from the yolk, and the first has divided transversely; in k (front view), the second is likewise dividing transversely, and the nucleus of the third giving rise to a new one; in l, II has completely divided, and III is cut off from the yolk; in m, the blastoderm now covers a good part of the yolk, a new blastomere is forming, and the nucleus is dividing, one daughter-nucleus being in the yolk; in n, the blastopore is being closed by the emergence of a merocyte; in o, this is dividing to form the last blastodermic cell, and complete the epiblast; in p, the blastoderm is

The segmentation is diagrammatically represented by the woodcuts fig. 3, a, b, c, &c. Figs. c and d show the concentration of the protoplasm at the anterior end. As the first blastomere becomes cut off from the yolk the nucleus divides (e, f) and one

daughter-nucleus passes into the yolk half, and soon emerges (*g*) accompanied by protoplasm to form a second blastomere and generally situated close to the first.   As

Fig. 3—(continued).
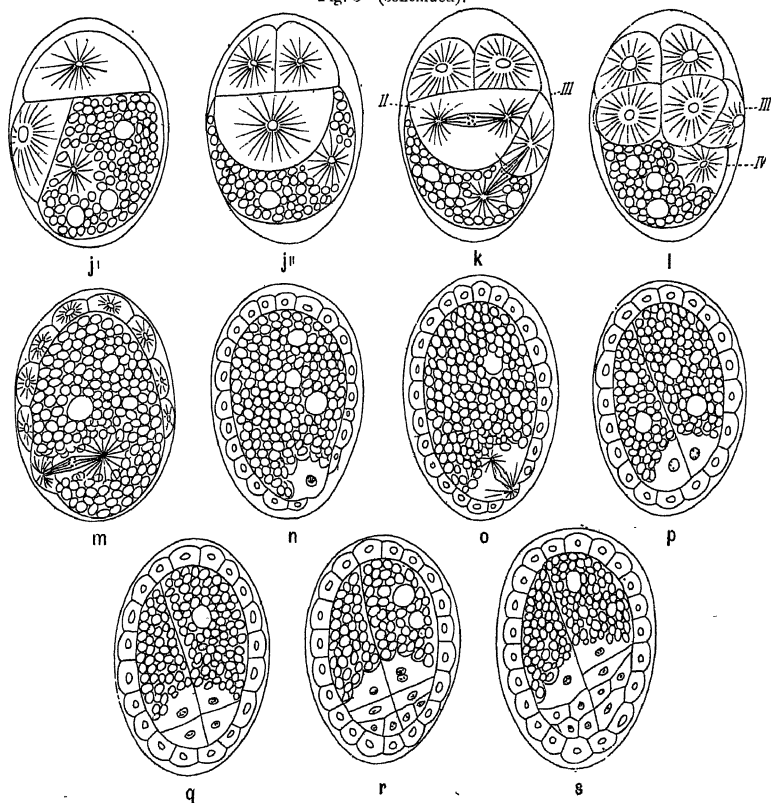
completed, and the nucleus left in the yolk has divided in two, and with it the whole yolk; in *q*, the two yolk-cells thus formed have cut off two mesoblastic cells at their hinder ends; in *r*, these latter have divided, and two new mesoblastic cells have been cut off from the yolk-cells; in *s*, the germinal layers are complete, the mesoblast occupies the hinder end, and the two yolk-cells, with their posterior nucleated protoplasmic masses the anterior end; the epiblast covers the whole.   (*m . . . s* are side views.)

this becomes cut off from the yolk it gives off (*h*) into the yolk a nucleus, which, behaving (*j, k, l*) similarly to the daughter-nucleus of the germinal vesicle, forms new protoplasm and emerges as a third blastomere.   At each successive stage the

yolk is in communication with one merocyte or newly-forming blastomere, and this, before becoming shut off as a blastomere, gives off a single nucleus into the yolk.

The yolk may be, it will be seen, regarded as a single cell with abundant nutritive material (and at times very little protoplasm), the nucleus of which by its division and creation of new protoplasm from the yolk granules forms new cells at its surface, and moves about from place to place in order to invest the yolk with a covering of blastomeres: the position of the nucleus (or its spindle) always more or less peripheral, and the idea of a central nucleus giving off nuclei radially, would not quite meet the case.

The mode in which the blastoderm grows round the yolk agrees essentially with the process of epiboly as defined by LANG (79). The definite endoderm is not formed till late, but the yolk may be regarded as a generating cell giving off in succession epiblastic cells which themselves divide up and tend to complete the epiblastic investment.

The circumstance that the yolk thus represents a single cell will exclude, I think, the view suggested by KORSCHELT and HEIDER (81) that the Cirripede ovum segments superficially during the later phases of cleavage.

This process may rather be compared to that taking place in the case of *Bonellia*, the four macromeres of this type being represented by a single one in the case of Cirripedes.

It is worthy of note that the yolk never appears to be cut off from all communication with the protoplasm in the way stated by some observers in the case of certain other Crustacea; one of the peripheral micromeres, or one of the merocytes derived from the latter, is always in communication with it, and it is, therefore, not to be regarded as extra-cellular. Nor is it to be regarded as belonging, from the first, solely to the hypoblast, for until the closure of the blastopore epiblastic cells are formed at its expense, and afterwards for a time also the mesoblast.

The effect of the nucleus in the transformation of yolk material into protoplasm is very marked in the Cirripede ovum. The yolk itself, throughout the main portion of its mass, contains very little protoplasm. All stages can be traced between a condition in which a nucleus, only recognizable by a slight stellate arrangement of the smaller granules with which it is surrounded, occurs near the micromere or emerging merocyte from which it has apparently been given off, to a well-formed micromere with abundant protoplasm. The radial arrangement becomes more definite by the continued transformation of the large yolk granules and oil globules into granular protoplasm, the granules of which become more and more distinctly arranged in radial rows: the area of transformation gradually extends, and eventually a considerable section of yolk is bodily transformed into protoplasm. When once this is seen it is very evident how all the primitive micromeres have arisen in this manner. The nucleus, accompanied as it is in most cases by little or no protoplasm as it passes into the yolk, evidently possesses this transforming power.

In describing the details of division of the cells of the blastoderm and yolk-endoderm much variation has been shown to occur, so much indeed that the process may be termed irregular. Such differences show well the morphological insignificance of the details of cell division in the present case, for the Nauplii vary proportionately much less; every one of the numerous, simple, or compound bristles or spines of the Nauplius has its definite character and position, which are maintained with surprising constancy throughout, although they must have been produced by epiblast cells having very different modes of origin and arrangement.

(B.) DIFFERENTIATION OF THE GERMINAL LAYERS.

With respect to the origin of the mesoblast and hypoblast of the Nauplius, the Cirripedes occupy an isolated position among Crustacea.

They differ from *Cetochilus* (35) among Copepods, *Moina* (65) among Phyllopods, and from *Astacus* (73), and most other Decapods, as well as from *Ligia* among Isopods (81), in the fact that the hypoblast and mesoblast do not arise as multicellular areas forming a specially differentiated and separate portion of the blastoderm, but from a common source, the single yolk cell, the nucleus of which is derived from the last, or possibly, in rare cases, from one of the last formed cells completing the blastoderm and filling the blastopore.

The mode in which the mesoblast and hypoblast are differentiated, presents most resemblance among Decapods to that found in *Palæmon* and *Eriphia*, where the mesoblast arises from the invaginated portion of the blastoderm, which also forms the mid-gut (and hind-gut). In *Oniscus* and *Cuma*, too, a simple blastodermic proliferation gives rise to both mesoblast and hypoblast, and the same appears to be true of *Daphnia* (81). The case of *Cyclops* may also be mentioned in this connection. Here the endoderm is stated to be derived by invagination (64), or from a single cell (67), which also possibly gives rise to the mesoderm; the mesenchyme cells, however, which give rise to the muscles of the Nauplius, are stated by URBANOWICZ to be derived from the ectoderm. The process in these cases may be regarded as representing an invagination of the meso-hypoblast, and that seen in Cirripedes may be looked upon as the same process reduced to its simplest expression, viz., a single cell (the uni-nucleated yolk) representing the common origin of both mesoderm and endoderm.

This cell divides immediately into a more dorsally and a more ventrally situated cell; from the hinder end of each of these cells uni-nucleated segments are cut off to form the mesoblast. When this process is completed, the yolk still consists of two cells; these represent the whole of the endoderm; behind this is a plug of mesoblast cells. The hypoblast then, at the time of its differentiation from the mesoblast, consists of two cells, while the mesoblast is multicellular.

The two protoplasmic bodies of the yolk-endoderm cells may be compared with the

amœboid cells at first bounding the archenteron in *Palæmon*, and probably with the cells with processes radiating into the yolk, and situated at the end of the invaginated portion of the blastoderm in *Eupagurus*.

The mode of origin of the mesoblast clearly shows that no paired pole-cells (teloblasts) such as have been supposed by GROBBEN to give rise to the mesoblast in *Balanus* and *Peltogaster* (35), are present.

No reproductive cells, such as are described in *Moina* and *Cetochilus*, can be recognized at an early period in Cirripedes.

In the circumstance that the blastopore closes completely, Cirripedes agree with *Moina, Cetochilus,* and *Cyclops;* it differs, however, possibly in position from that of *Moina*, where it is supposed, by GROBBEN, to close on the site of the future mouth. It resembles that of the majority of Crustacea in showing no extension in the direction of the mouth.

(C.) FORMATION OF THE ALIMENTARY CANAL.

The yolk-endoderm gives rise to the stomach (with its glands) alone of the Nauplius and adult Cirripede.   As the mesoblast develops into muscles and connective tissue, the central part of the yolk is absorbed, the nucleus retiring to the periphery; in this way the hollow mesenteron is formed, the walls of which are finally composed of a number of clear nucleated endoderm cells devoid of yolk, each formed by the centrifugal contraction of a yolk-pyramid.   The yolk-pyramids are essentially similar in structure, relations, and fate to the secondary yolk-pyramids of *Astacus* (REICHENBACH'S "Secundäre Dotterpyramiden"), and are evidently the equivalents of the latter.

(D.) ORIGIN OF THE NAUPLIUS APPENDAGES.

A striking feature of the mode of origin of the Nauplius appendages is that they appear first on the dorsal side of the embryo.   It is only, however, the free ends which are thus seen, the main part of the appendage being applied to the sides of the body, and the origin, as usual among appendiculate animals, ventral.

In Cirripede groups other than Thoracica a similar mode of origin occurs : VAN BENEDEN (58) has already described the same thing in *Sacculina*, and I have observed it myself in *Peltogaster*.   A similar disposition of the Nauplius appendages in *Laura* has caused LACAZE-DUTHIERS (38) to state that the appendages are attached on the dorsal side, a peculiarity he specially emphasizes.   The attachment is, I believe, ventral as usual, but the free ends are dorsally directed, the position of the embryo having been inverted by this observer.

It seems very probable that the same fact obtains in the Nauplii of all the other groups.

In the Phyllopods the figures of GROBBEN make this clear, and he states, for *Moina*, "Sowohl die beiden Antennen als die Mandibel wachsen von innen nach aussen."

In *Cetochilus* the same author failed to follow the origin of the appendages, but says, "Nachdem sich die auf die Dorsalseite zurückgelegten Extremitäten deutlicher entwickelt haben. . . ."

In *Anchorella*, *Lernæopoda*, and *Brachiella*, VAN BENEDEN (60) states that the Nauplius appendages develop from within outwards.

The same is apparently true of *Tracheliastes polycopus* (63) and *Argulus foliaceus* (62), as well as *Nebalia* (68).

In *Mysis ferruginea*, VAN BENEDEN (70) figures and describes a similar disposition of the Nauplius appendages.

In Decapoda, Amphipoda, and Isopoda, where the Nauplius stage is represented but concealed, the Nauplius appendages likewise grow dorsally, as follows from the descriptions or figures of REICHENBACH (73), FAXON (72), KINGSLEY (74), and others, for the Decapoda (*Astacus, Palæmonetes, Crangon*), and from the figures of RATHKE (75) for Isopoda and Amphipoda (*Bopyrus, Amphithoe*), and of VAN BENEDEN for *Asellus* (76).

This mode of development holds good, therefore, in all probability, for the Nauplius or Nauplius-stage of Phyllopoda, Cirripedia, Copepoda, Leptostraca, Schizopoda, Decapoda, and Thoracostraca (Amphipoda, Isopoda).

As early as 1869, VAN BENEDEN indeed drew attention to the generality in the difference in the mode of development of the Nauplius appendages and the later ones, and, speaking of *Asellus*, says, "Ses organes se développent rapidement, et s'allongent en se portant en arrière et en dehors. Il parait en être de même chez tous les Crustacés. Partout les antennes et les mandibules semblent se développer de dedans en dehors, et, par là, les trois premières paires d'appendices se distinguent de tous les autres, qui se développent, au contraire, de dehors en dedans, en se rapprochant de la ligne médiane."

This law, however, has been generally overlooked, not only in Cirripedes, but also in Copepods; and it appears almost certain that in the latter group, as well as in the former, the surface of the embryo, on which the median longitudinal and transverse furrows appear, and which has been described as ventral, is in reality dorsal.

The dorsal position of the mesoderm plate is evidently in relation with the dorsal position of the appendages, for a considerable part of the mesoderm goes to form the latter. URBANOWICZ (67) describes a similar accumulation of his "mesenchyme" cells on the dorsal side in *Cyclops*, and states that they form the muscles of the appendages.

(E.) BODY CAVITY.*

Inasmuch as the body cavity arises partly owing to the more rapid growth of the ectoderm and the endoderm, and consequent separation of these layers, it might be

* This term is used in a purely descriptive sense, to denote generally the space included within the body walls.

termed a blastocœle; but since mesoderm cells exist between the two layers, and upon separation of these remained, some associated with the ectoderm, some with the endoderm, and some spanning the space intervening between the two, it may be said with equal truth to be a schizocœle, and regarded as arising by a failure of the mesoderm to keep pace with the growth of the layer bounding it, and its consequent splitting or excavation by the formation of vacuoles.   If no mesoderm were present at the stage when the cavity arose, it could be termed a blastocœle; if a complete mesodermic layer were present and split into somatic and splanchnic layers, it would be termed a schizocœle; but since this layer is not complete, and a true split does not occur, the body cavity may be said to be in part a schizocœle and in part a blastocœle.

The mesoderm of the Nauplius shows no trace of an arrangement into somites, and the body cavity is continuous from one end of the body to the other ; the mesoderm thus resembles in its deportment that of the cephalic extremity in *Astacus*.

(F.) Nervous System.

The nervous system arises, probably, mainly or altogether as an epiblastic thickening, and at Stage 2 retains everywhere its primitive connection with the ectoderm ; it has ganglion cells along its whole extent.

In other respects the nervous system shows much specialization.   It is sharply marked off from the rest of the ectoderm, and is distinctly divided into ganglia and connectives.   The commissures are practically absent, and so far from showing ganglia corresponding to the metameres indicated by the appendages, the nervous system is considerably complicated, even at this early period.   It shows, however, an anterior portion, which is in close connection with the frontal filaments and Nauplius eye, and may be regarded as corresponding to the archi-cerebrum of LANKESTER.

In Balanids two accessory and a central lobe of unknown significance are present, as well as short thick nerves passing to the labrum.

The two accumulations of ganglion cells situated just at the commencement of the circum-œsophageal connectives, and coming into close relation with the antennules, probably correspond to the ganglia of the first post-oral somite, and inasmuch as these cells belong to the brain, the latter may be said to be a syn-cerebrum from the first, though its component elements, the archi-cerebrum and ganglia of the antennules, may still be recognized.

The ganglion cells on the circum-œsophageal connectives which come into close relation with the origin of the antennæ probably represent the ganglia of the second post-oral somite, while the large sub-œsophageal ganglion represents the fused ganglia of the mandibles.

(G.) Morphology of the Appendages.

Comparing the three pairs of appendages, the antennæ and mandibles are seen to be very similar in structure. Each consists of a two-jointed protopodite bearing inwardly directed plumose spines; the endopodite has a series of stiff bristles or spines forming a series continuous with those of the protopodite; the bristles are given off generally in pairs, while the distal end of each joint of the much more jointed exopodite gives off a single bristle.

The single ramus of the antennules resembles the protopodite and endopodite together more closely than it would the protopodite and exopodite, the joints being few and the bristles given off singly, or in groups of two or four; one bristle is given off from the middle of the third joint like the two or three on the first joint of the endopodite of the antennæ and mandibles.

It is therefore probable that the appendages are serially homologous, and that it is the exopodite of the antennules which is absent.

That the antennules are not of a different nature to the two remaining pairs of appendages is also indicated by the similar and peculiar origin of all three.

I believe, therefore, that all the appendages are of the same kind, and may represent, as Lankester contends, for Crustacea generally (66) primitively post-oral appendages.

It is important to remark that the first two pairs of appendages are, however, never ontogenetically post-oral, the antennules being from the first in front of the mouth, and the antennæ at its sides; this, however, together with the pre-oral innervation, may be one of the many signs of early specialization visible in the Nauplius, such as have led to the view that the Nauplius is a trochosphere with precocious Crustacean characters (79).

(H.) Comparison of the Nauplii of the Different Species.

(α.) Points of Agreement.

In the general description it has already been seen how closely the Nauplii of all Thoracic Cirripedia resemble each other. Of these points of agreement we may distinguish—

(i) Characters shared by all the Nauplii.

(ii) Special characters of the Cirripede Nauplius.

Though until the Nauplii of the various sub-divisions of Crustacea have been properly examined it will be impossible to assign all the characters to one or other of these divisions, the striking features of the Nauplius are well known, and need not be repeated here.

Among the peculiar features of the Cirripede Nauplius the following are the most important:—

1. The shape of the carapace with its fronto-lateral horns and caudal spine.
2. The presence and structure of the fronto-lateral glands.
3. The size, shape, and structure of the labrum.
4. The structure of the axial gland of the labrum.
5. The character of the setose region.
6. The structure of the appendages.
7. The size and form of the tail (thorax-abdomen).

The agreement of the Nauplii of genera so distinct as *Lepas* and *Balanus*, as well as of Nauplii more or less intermediate between these types, such as *Chthamalus*, in these particulars is highly remarkable and significant. The perfect similarity which obtains in all the species I have examined in the number, disposition, and minute character of the very numerous bristles and other processes on the appendages of Nauplii of the second stage—a similarity which cannot be supposed to be merely analogical—demonstrates that the character of the appendages is a primitive one, actually possessed by the common ancestor of the Thoracica at some stage in its life history.

The majority of the characters common to all the Nauplii being shown in this way to be ancestral, it becomes necessary to determine whether the Nauplius of the Thoracica represents in any sense their ancestor, the former adult characters being supposed to have been precociously acquired by the larva, or whether the features in question belonged to a Nauplius possessed by the ancestor.

Now the structure of the adult *Lepas* and *Balanus* is so obviously similar that we can only suppose the two to have diverged from a similar ancestor. The ancestral adult did not therefore possess the peculiar features of the Nauplius.

The correspondence between the two forms at other stages is so close that we can only suppose the essential features of each stage to be ancestral; thus the segmentation of the ovum, the peculiar mode of formation of the embryo—so similar (as I have shown) in all the species—must have been shared by the ancestor of the group. Of the Cypris stage DARWIN says (10): "In the pupæ of all these genera (*Lepas, Conchoderma, Dichelaspis, Ibla, Alcippe,* and *Balanus*) there is a most close general agreement in structure, excepting in minute detail. I was surprised to find exactly the same slight differences in the spines on the first pair of natatory legs, as compared with the succeeding pairs, in *Balanus hameri* as in *Lepas*."

We cannot escape from the conclusion that the very similar development in *Lepas* and *Balanus* (which I hope to illustrate in a further communication)* corresponds stage for stage with that of the ancestor of the Thoracica, or in other words, that the

* I may state that before reaching the Cypris stage *Balanus perforatus* undergoes five moults; *Lepas fascicularis* probably undergoes the same number, though owing, apparently, to inadvertence the number is said by WILLEMOES-SUHM (28) in one place to be seven. The six Nauplius stages correspond, probably, exactly. The agreement of the first two stages in *Balanus, Chthamalus, Conchoderma,* and *Lepas* in particular detail has been shown above.

latter underwent a metamorphosis perfectly similar to that of the present members of the group.

It will not be difficult to test this conclusion, for a careful study of the affinities of the Thoracic Cirripedes indicates that the Balanids, on the one hand, have probably diverged from *Pollicipes*, and the majority of the Lepads, on the other, from *Scalpellum;* these two genera are connected, as DARWIN and HOEK have shown, by intermediate forms. The ancestor of the whole group has therefore been preserved probably in a very slightly altered form, and it will be possible to study its development in detail. As far as an approach has been made to this, the results confirm the above conclusions ; *Scalpellum* apparently, from the descriptions of HESSE (12, 25), passes through a metamorphosis similar to that of *Balanus* and *Lepas*, and the segmentation of the ovum, as figured by LANG in *Scalpellum*, and NUSSBAUM in *Pollicipes*, does not differ from that of the other Thoracica, while the larva in the latter genus is a typical Thoracic Nauplius.

The permanence of such minute characters as the arrangement of the bristles on the appendages for the vast time represented by the Tertiary, Cretaceous, and probably, at least, part of the Jurassic periods, is highly remarkable, and well shows the slow rate of evolution which may take place in so highly specialized a group.

(β.) *Larval Differences.*

In spite of the great agreement of the different Nauplii in many points, there are nevertheless well-marked differences by which the Nauplii of all the genera examined, and often of the species, can be readily determined.

These points of difference relate to—

1. Size.
2. Shape of the carapace.
3. Length of the fronto-lateral horns.
4. Length and character of the caudal spine.
5. Shape and size of the labrum.
6. Form of the axial gland of the labrum.
7. Character of the setose region.
8. Slight modifications in the structure of the appendages, and in the relative proportions of their parts.
9. Length and character of the tail.
10. Extent of " ventral plate."
11. Structure of the nervous system.
12. Presence or absence of lateral glands.
13. Presence or absence of certain connective tissue elements.
14. Physiological properties—Heliotropism—Rapidity of motion.

In the following Tables a classification of the Nauplii according to their resemblances, and perfectly independent of conclusions drawn from the adult, is given.

(γ.) *Classification of Cirripede Nauplii of Stage 2.*

Lepas, Conchoderma, and *Dichelaspis* (Lepadidæ).

   i. Nauplii long (0·6 to 0·8 millim.), slender and transparent; movements slow (at least in the first two genera).

   ii. Caudal spine long and covered with secondary spinelets.

   iii. Labrum long; proximal lobe ovate, distal relatively large and pentagonal; axial gland well developed in both divisions of labrum.

   iv. Tail long, with secondary spinelets; ectodermal thickening represented by two small groups of cells.

   v. Brain two-lobed (accessory lobes absent).

   vi. Lateral glands absent.

   vii. All the bands of the setose region present (*Dichelaspis ?*).

(A.) *Lepas* and *Conchoderma.*	(B.) *Dichelaspis.*
(α.) Carapace large, shield-shaped.	(α.) Carapace narrow, triangular.
(β.) Fronto-lateral horns long and slender.	(β.) Fronto-lateral horns of moderate length.
(γ.) Distal lobe of labrum broad, with one large median and two lateral teeth; axial gland narrow distally.	(γ.) Distal lobe of labrum narrow, with one large median tooth; axial gland dilated distally.
(δ.) Maxillary band of setose region well developed.	(δ.) Setose region ?
(ε.) Ova blue, relatively broad.	(ε.) Ova vermilion-red, narrow (*D. Darwinii*).

Lepas.	*Conchoderma.*
Tail simple.	Tail bifid.

L. fascicularis, length 0·6 mm.
L. pectinata   „   0·7 „
L. anatifera   „   0·8 „

Balanus and *Chthamalus* (Balanidæ).

   i. Nauplii of moderate length (0·23 to 0·46 millim.); movements rapid.

   ii. Caudal spine of moderate length, with very short secondary spines; fronto-lateral horns short or of very moderate length.

   iii. Labrum shorter and broader than in Lepadidæ; proximal lobe broad; distal lobe relatively small and rounded; axial gland developed almost exclusively in the distal portion (*Chthamalus ?*).

   iv. Tail short.

v. Brain complex (accessory lobes present).

vi. Lateral glands present.

vii. Maxillary band of setose region as yet absent.

(A.) *Balanus* (Balaninæ).	(B.) *Chthamalus* (Chthamalinæ).
(α.) Carapace shield-shaped, with two small lateral teeth.	(α.) Carapace almost circular.
(β.) Tail and caudal spine with minute teeth; ectodermal thickening of tail a considerable plate on each side.	(β.) Tail and caudal spine with strongish spinelets, especially at sides of base of latter; ectodermal thickening of tail a few cells on each side.
(γ.) Proximal lobe of labrum with two small lateral lobes; distal lobes small, with two lateral teeth.	(γ.) Proximal lobe of labrum simple; distal with two lateral, several central, and smaller teeth.
(δ.) Pre-maxillary bands slight; anterior band complex; extra-maxillary are slight.	(δ.) Pre-maxillary bands strong and united together; anterior band and post-oral group ?; extra-maxillary are distinct.
(ε.) Ova brown; yolk granules large; oil globules small.	(ε.) Ova orange coloured, narrow; yolk granules small; oil globules large.

B. perforatus.	*B. improvisus.*	*B. balanoides.*
Length 0·46 mm.	Length 0·27 mm.	Length 0·45 mm.
Horns moderately short.	Horns moderately short.	Horns short.
Tail rather short.	Tail rather short.	Tail short.
Caudal spine rather short.	Caudal spine rather short.	Caudal spine longish.

These tables illustrate the differences which may obtain among the larvæ of allied animals.

We see that, among Cirripedes, the larvæ and embryos of all the genera differ not inconsiderably. The embryos of *Lepas*, *Conchoderma*, or *Balanus* can be distinguished at any stage by one or more features, and the Nauplii much more readily. The species, or even in some cases the genera are not so readily recognized, for though, in *Lepas anatifera* and *L. pectinata*, the ova and embryos can with care be distinguished by the size and shape, yet the same stages of *Conchoderma virgata* and *Lepas anatifera* can hardly be distinguished at any stage, and even the Nauplii can only be separated by the slight fork in the tail of the former.

The differences between the species go back as far as the new laid ovum, as may be seen from the sections on the size, shape, colour, and constitution of the ova. Thus the narrow orange-coloured ova of *Chthamalus* with their large oil drops may be distinguished from the broader yellowish ones of *Balanus* with their more numerous but smaller oil globules, and from the smaller vermilion eggs of *Dichelaspis*, and from the broader blue ova of *Lepas* and *Conchoderma*. The ova of *Lepas pectinata* are

narrower than those of *L. anatifera* and *Conchoderma virgata.* In the genus
Balanus the ova of the three species *B. improvisus, B. perforatus,* and *B. balanoides*
differ much in size, the first-mentioned having the smallest and the last the largest
ova, measurements of which have been given. Sections of *Lepas* and *Balanus,* and
probably of *Chthamalus,* at any stage of embryonic development can be distinguished
by the characters of the yolk. It must be stated, however, that the specific and even
generic differences (*cf. Lepas anatifera* and *Conchoderma virgata*) may not be
recognized in single specimens, and that it may be necessary to compare a number
of cases in order to eliminate the effect of variation.

The Nauplii at the second stage can be readily classified and might, in most cases,
be used for the formation of families, genera, and species. The differences certainly
are not very great, but seem to be as important relatively to the Nauplii as the
generic differences are to the adult.

It is instructive to observe that the classification of the genera as deduced from the
developmental stages agrees perfectly with that arrived at from the relations of the
adult : the differences between the Nauplii of the different genera are approximately
proportional to those between the corresponding adults ; thus the two genera most
widely remote from one another, *Lepas* and *Balanus,* also show the greatest differ-
ences in their Nauplii. The Chthamalinæ are those members of the Balanidæ which
approach the Lepadidæ most closely, and their Nauplii show similar affinities. *Lepas*
and *Conchoderma* are perhaps, in some respects, the highest members of the
Lepadidæ, and their Nauplii show, in some respects, more special features than those
of *Dichelaspis,* which branched off from the main stem at an earlier period. The two
former genera so closely allied in the internal organization have likewise Nauplii so
much alike as to be almost indistinguishable.

(δ.) *Larval Evolution.*

The phylogeny of the Cirripedes, as deduced from the adult anatomy, may be
represented as follows :—
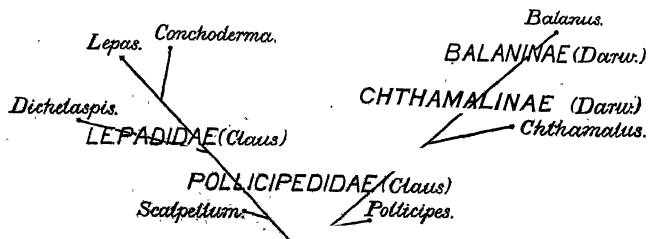
These Nauplii fit in well with this scheme ; they have undergone an evolution

parallel to and simultaneous with that of the adults, and the results obtained in each case are nearly proportional.

It is a point of some interest to inquire whether the new characters assumed by the larvæ have been acquired independently, or are due to a precocious appearance of features properly belonging to the adult, in the way indicated by FRITZ MÜLLER (16) and WEISMANN (79), or whether both processes have taken place.

Glancing down the list of larval differences given on pp. 205–7, it is evident that though some of the characteristic differences may possibly be due to precocious acquirement by the young Nauplius of the forms in question of characters originally common to older larvæ at an earlier phylogenetic stage, such as the early acquirement of glands (the lateral glands) in Balanids possibly properly belonging to the Archizoæa stage in Lepads, yet few of the characters can be conceived as transferred back from even a young sessile form ; most of the differences affect structures peculiar to the Nauplius, and lost by the adult.

It is thus evident that the variation in the Nauplii (the same may be said of the ova and embryos), though always accompanying adult variation, has taken place in a perfectly distinct direction.   Differences in the length of the horns, in the length and armature of the caudal spine, in the character of the setose region, or in the presence or absence of the lateral glands must have been produced in this way.

Few probably of the other characters can be referred to larval precocity.   Though the species which have the smaller sized Nauplii have as a rule the smaller sized ova* (see table, p. 130), yet in neither case is the size proportional to that of the adult ; thus, though the Nauplius of *Lepas anatifera* is larger than that of the smaller *L. pectinata*, the latter has a Nauplius larger than that of *L. fascicularis*, which is about twice the size.   Similarly, the small *Balanus balanoides* has a Nauplius nearly twice the size of that of the perhaps rather larger *B. improvisus*.   The labrum of the Nauplius, with its axial gland, is practically lost at the Cypris stage, and that of the sessile Cirripede differs considerably from the earlier structure.   The greater complication of the brain in the Balanids may be a precocious character, but until the brain of the Nauplius and of the adult have been compared this, too, is uncertain.   The early appearance of the thickening on the tail, which gives rise to important parts of the body of the thorax in *Balanus*, is the only character which appears definitely to indicate the precocious appearance of features originally belonging to a later ontogenetic stage.

The origin and meaning of the Nauplius hardly come within the scope of the present subject, since, as MARSHALL (80) has pointed out from other considerations, the origin of this larval form is outside the group.

I must, in conclusion, express my best thanks to Messrs. SEDGWICK, HARMER, and WELDON, at whose suggestion I undertook the study of the Cirripedes, and who have

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* The shape, too, is to a certain extent adapted to that of the Nauplii, *cf. Lepas, Dichelaspis,* and *Balanus.*

frequently assisted me with their advice; to Professor WELDON I am specially indebted for many valuable criticisms. My warmest thanks are also due to Dr. EISIG, who showed me much kindness, and assisted me in every way possible during my stay at the Zoological Station at Naples. To Dr. PAUL MAYER I am indebted for the specimens of the Nauplii of *Dichelaspis,* and for information kindly given me on certain of his methods. To Professor KOWALEVSKY I am indebted for suggestions as to the best method of investigating the process of excretion in the Nauplii. To my friend Dr. HOEK for the kind way in which he has assisted me by the gift or loan of literature treating of Cirripedes; and lastly, I have to thank Signor LO BIANCO for the ready way in which he provided me with the large amount of material necessary for embryological study, and Professor MIALL for affording me facilities for completing my work in the laboratory of the Yorkshire College.

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DESCRIPTION OF PLATES.

EXPLANATION OF LETTERING.

abd.reg.	Abdominal region.	*frl.gl.s.*	Secretion of ditto.
an.arc.	Anal arc.	*frl.h.*	Fronto-lateral horn.
an.dil.	Dilator of anus.	*gn.*	Gnathobase of antennæ.
ant.[1]	Antennules.	*gn.m.*	Muscle to ditto.
ant.[2]	Antennæ.	*gr.mat.*	Granular matter.
anus.	Anus.	*gt.c.m.*	Circular muscles of stomach
app.	Appendage.		and intestine.
app.d.musc.	Dorsal muscles to appen-	*gt.l.m.*	Longitudinal muscles of
	dages.		ditto.
app.v.musc.	Ventral muscles to appen-	*int.*	Intestine (proctodæum).
	dages.	*lat.gl.*	Lateral gland.
arch.	Archenteron (stomach).	*lbr.*	Labrum.
ax.gl.	Axial gland of labrum.	*lbr.br.*	Bristles on labrum.
ax.gl.n.	Nucleus of ditto.	*lbr.c.*	Cells at sides of labrum.
ax.gl.fi.	Fibre to axial gland.	*lbr.dist.*	Distal lobe of labrum.
b.c.	Body cavity.	*lbr.n.*	Nerve to labrum.
bl.	Blastopore.	*lbr.prox.*	Proximal lobe of labrum.
br.	Brain (anterior lobes).	*mer.*[1], *mer.*[2], &c.	Merocytes.
br.acc.l.	Accessory lobes of brain.	*mes.c.*	Mesoderm cells.
br.c.l.	Central lobe of brain.	*mes.'*	Mesoblast of Nauplius.
br.p.l.	Posterior lobes of brain.	*mnd.*	Mandibles
car.	Carapace.	*mo.*	Mouth.
c.sp.	Caudal spine.	*mx.arc.*	Maxillary arc.
c.o.c.	Circum-œsophageal connec-	*m.pn.*	Male pro-nucleus.
	tives.	*Npl.eye.*	Nauplius eye.
c.t.	Connective tissue.	*œs.*	Œsophagus (stomodæum).
d.b.	Dorsal body.	*œs.d.m.*	Dilator muscles of ditto.
ect.	Ectoderm.	*o.g.*	Oil globules.
ect.n.	Nuclei of ectoderm.	*pmx.bd.*	Premaxillary band.
end.	Endoderm.	*ppm.*	Protoplasm of blastoderm.
ex.m.arc.	Extra-maxillary arc.	*proct.*	Intestine (probably = proc-
fl.arc.	Flexor arc.		todæum).
fl.ta.	Flexor of the tail.	*pv.*[1]	First polar body.
fr.fil.	Frontal filaments.	*pv.*[2]	Second polar body.
fr.fil.bs.	Base of ditto.	*st.*	Stomach.
frl.gl.	Fronto-lateral gland.	*st.gl.c.*	Glandular cells of stomach.
frl.gl.n.	Nucleus of ditto.	*st.b.*	Stellate bodies.

stom.	Œsophagus (probably = stomodæum).	*ves.t.*	Vesicular tissue.
		vt.m.	Vitelline membrane.
sub-œs.g.	Sub-œsophageal ganglion.	*yk.*	Yolk.
ta.	Tail (thorax-abdomen).	*yk.end.*	Yolk-endoderm.
ta.th.	Ectodermal thickening in thorax-abdomen (ventral plate).	*yk.end.c.*	Yolk-endoderm cells.
		yk.end.n.	Yolk-endoderm nuclei.
		yk.gr.	Yolk granules.
ta,sp.	Strong pair of spines on tail.	*yk.n.*	Nuclei of meso-hypoblast cells.
th.reg.	Thoracic region.		

PLATE 14.

LEPAS ANATIFERA. (Figs. 1–19.   × 300.)

Stage A.

Fig. 1. Unfertilized ovum, just laid.

Fig. 2. Ovum just after giving off the first polar body (*pv¹.*).

Fig. 3. Ovum undergoing retractions within the newly-formed peri-vitelline membrane.

Fig. 4. Ovum just after giving off the second polar body (*pv².*).

Fig. 5. Ovum during the process of separation of the yolk and protoplasm, showing the commencing formation of the first blastomere and central mass of protoplasm.

Fig. 6. Ovum showing the first blastomere clearly defined peripherally by a sharp line, while, internally, it is still in connection with the central mass of protoplasm. No nucleus is visible without preparation.

Fig. 7. Somewhat later stage in which the segmentation-nucleus has become visible as a clear round spot. The first blastomere is a larger one than that of fig. 6.

Stage B.

Fig. 8. Ovum in which the first blastomere is now clearly marked off by a constriction, and in which the nucleus has divided. One of the daughter-nuclei is passing towards one side of the central mass of protoplasm, which is still in communication with the protoplasm of the first blastomere.

Fig. 9. Embryo in which the first blastomere has become definitely cut off from the yolk by a transverse wall. One of the daughter-nuclei of the segmentation nucleus has passed to the periphery of the yolk, where new protoplasm is forming at the expense of the latter, the central mass of

protoplasm having likewise become peripheral. The protoplasm of the newly-forming second blastomere is seen to be still in connection with the yolk.

Fig. 10. Similar stage in which the basal plane was oblique.

Fig. 11. Similar stage in which the first blastomere is not marked off by a constriction. The nuclei are large and clear.

Fig. 12. Lateral view of a stage in which the second blastomere has become pretty clearly defined peripherally. Both blastomeres (separated by an oblique basal plane in this case) are dividing transversely.

Fig. 13. Stage in which the second blastomere is arising at some distance from the first, which, in the meantime, is dividing transversely.

Fig. 14. Similar stage, but the second blastomere is in contact with the first, and its nucleus lies in the plane of division of the latter.

Fig. 15. Stage, with three blastoderm cells, and a fourth appearing on the left-hand side of II.

Fig. 16. Stage, with three blastomeres, and a fourth, nearly completed, on the right-hand side of II.

Fig. 17. Stage, with three blastomeres, and a fourth emerging beneath II. Ventral view.

Fig. 17A. Stage, with three blastomeres, and two new ones arising by division of a merocyte before it emerges from the yolk. Ventral view.

Fig. 18. Stage, with six blastomeres, one terminal (anterior), two dorso-lateral, two ventro-lateral, and one approximately ventro-lateral (dividing).

Fig. 19. Stage with seven blastomeres, two dorso-lateral, four ventro-lateral, one ventral, and one emerging ventro-laterally.

PLATE 15.

LEPAS ANATIFERA. (Figs. 20–39. × 300.)

Fig. 20. Lateral view of stage with fifteen cells, one dorsal, four dorso-lateral, six lateral, and four ventral.

Fig. 21. Stage in which the yolk is largely covered by the blastoderm; blastopore still large. The covered part of the yolk is shaded.

Fig. 22. Further stage; blastopore quite small (the yolk seen to project through it near the posterior end on the right-hand side). The yolk is faintly shaded.

Fig. 23. Stage in which the blastopore is closed by the formation of a new blastoderm cell over the uncovered part of the yolk. The yolk (which is shaded) is still undivided.

Stage C.

Fig. 24. Stage in which the yolk (meso-hypoblast) has just divided by an oblique furrow.

Fig. 25. Stage showing the production of the mesoblast of the Nauplius from the hind part of the two meso-hypoblast cells.

Fig. 26. Stage with three endoderm cells.

Fig. 27. Stage with four endoderm cells.

Fig. 28. Side view of stage with six endoderm cells, showing commencing segmentation of the body into three regions.

Stage D.

Fig. 29. Ventral view of stage in which the body is clearly divided into three regions.

Fig. 30. Same stage, side view. The furrows are seen to die out on the sides of the body.

Fig. 31. Same stage, dorsal view.

Stage E.

Fig. 32. Stage in which the three pairs of appendages are first marked out. Ventral view. $Ant.^1$, antennule; $ant.^2$, antennæ; $mn.$, mandible; $ta.$, tail.

Fig. 33. Same stage, lateral view.

Fig. 34. Dorsal view of same stage, showing the free ends of the appendages meeting in the mid-dorsal line.

Stage F.

Fig. 35. Ventral view of stage in which the appendages are well developed, but still short.

Fig. 36. Same stage, dorsal view.

Fig. 37. Lateral view of stage in which the appendages are longer, and directed obliquely backwards and upwards. $Ant.^1$, antennule; $ant.^2$ antennæ; $mn.$, mandible; $ta.$, tail; $lbr.$, labrum. The hinder end of the labrum is marked by a slight notch at the level of the letters $ant.^1$

Fig. 38. Stage in which the appendages are still of moderate length: the setæ becoming visible at the tips of the appendages. Ventral view. Lettering as in fig. 37. The tip of the labrum is seen opposite the middle of the antennæ: the endoderm is shaded.

Fig. 39. Lateral view of stage in which the appendages are a little longer. The endoderm is faintly shaded.

PLATE 16.

LEPAS ANATIFERA.

Fig. 40. Same stage as in fig. 39 : dorsal view. The boundaries of the endoderm cells are clearly seen. × 300.

Stage G.

Fig. 41. Dorsal view of a Nauplius approaching maturity, but still enclosed within the peri-vitelline membrane. *Yk.end.*, yolk-endoderm ; *c.sp.*, caudal spine ; *ta.*, tail ; *br.*, brain (the Nauplius eye is not yet visible) ; *frl.gl.*, fronto-lateral gland ; *frl.h.*, fronto-lateral horns.

Stage H.

Fig. 42. Dorsal view of a Nauplius removed from the peri-vitelline membrane shortly before hatching. *Arch.*, stomach ; *br.*, brain ; *c.sp.*, caudal spine, telescoped within the body ; *frl.gl.*, fronto-lateral gland ; *frl.h.*, fronto-lateral horn ; *gr.mat.*, granular matter ; *npl.eye*, Nauplius eye.

LEPAS PECTINATA.

Fig. 43. Ovum of *Lepas pectinata* after formation of the second polar body (pv^2).
Fig. 44. Ovum contracting during the process of separation of the protoplasm and yolk. The protoplasm is commencing to collect at the anterior end.
Fig. 45. Same ovum, five minutes afterwards ; a good deal of the protoplasm is now seen at the anterior end.
Fig. 46. Same ovum, thirty minutes later ; the segmentation-nucleus is now visible as a faint clear spot.
Fig. 47. Later stage of another ovum ; the first blastomere marked off by a deep constriction.
Fig. 48. Embryo in which the first blastomere was separated from the yolk by an oblique basal plane ; the second blastomere forming from the yolk.

BALANUS PERFORATUS. (Figs. 49–59.   × 265.)

Stage A.

Fig. 49. Ovum of *Balanus perforatus* in which the protoplasm has segregated from the yolk.

2 F 2

Stage B.

Fig. 50. Stage in which the first blastomere has been cut off from the yolk, and a second is arising from the latter.

Fig. 51. Stage in which the second blastomere is emerging, and the first dividing transversely.

Fig. 52. A similar, but somewhat later stage, in which the second blastomere is well marked off peripherally from the yolk.

Fig. 53. Stage with three blastomeres, and a fourth and fifth arising by division of a single merocyte. Another example was similar, but the merocyte was undivided.

Fig. 54. Stage with three blastomeres, the unpaired one (II.) dividing transversely.

Fig. 55. Dorsal view of stage with three blastomeres, a new one arising near the posterior end.

Fig. 56. Ventral view of similar stage.

Fig. 57. Stage with the blastomeres, two of which (probably all three*) are dividing ; a merocyte dividing in the yolk.

Fig. 58. Stage with four blastomeres ; a new one arising near the posterior end.

Fig. 59. Stage with four blastomeres, one (ventral) dividing transversely; a merocyte emerging on the right-hand side.

PLATE 17.

BALANUS PERFORATUS.   (Figs. 61–78.   × 265.)

Fig. 61. Stage with five blastomeres, a sixth arising from the yolk.

Fig. 62. Stage with seven blastomeres, one terminal, one ventral, two ventro-lateral, two lateral, and one dorsal ; an eighth arising from the yolk.   Ventral view.   The Roman numerals refer to the successive merocytes.

Fig. 63. Similar stage, ventral blastomere dividing.

Figs. 64–66. Later stages, showing further growth of blastoderm over yolk.

Fig. 67. Stage in which the blastopore is an irregular space becoming further diminished in size by the appearance of a new merocyte.

Fig. 68. Stage in which the blastopore is still smaller ; a new blastomere is arising at the apex of the projecting portion of the yolk.

Fig. 69. Stage in which the blastopore is very small.

Fig. 70. Stage in which the blastoderm is just completed by a merocyte emerging at the apex of the yolk to form a low blastomere just anterior to the most posteriorly situated cell.   The yolk is still undivided.

Fig. 71. Similar stage, in which the outline of the yolk is confluent with that of the cell which closed the blastopore.   The yolk still undivided.

_____
* I did not succeed in seeing the opposite side of this egg.

Stage C.

Fig. 72. Stage in which the yolk (meso-hypoblast) is divided in two.

Fig. 72a and 72b. Two lateral views of stages with four endoderm cells.

Fig. 73. Lateral view of stage with six endoderm cells.

Fig. 75. Ventral view of approximately same stage as that of fig. 73.   The nuclei of the endoderm cells appear as dark stellate spots.

Stage F.

Fig. 76. Lateral view of stage with short appendages.   *ant.*,[1] antennules; *ant.*,[2] antennæ ; *mn.*, mandibles ; *ta.*, tail.

Fig. 77. Dorsal view of stage in which appendages are rather short; the second and third pairs are bifid at their extremities.   Lettering as in fig. 76.

Fig. 78. Same stage, side view.   The nuclei of the endoderm cells may be sometimes seen dividing.   Lettering as in fig. 76.

PLATE 18.

BALANUS PERFORATUS.* (Figs. 79–82.   × 265.)

Fig. 79. Same stage as in fig. 78 ; ventral view.

Fig. 80. Stage in which the appendages are of moderate length ; labrum (*lbr.*) well-marked ; lettering as in fig. 82 ; ventro-lateral view.

Stage G.

Fig. 81. Lateral view of stage in which the appendages are long; Nauplius eye as yet absent; lettering as in fig. 82.

Stage H.

Fig. 82. Nauplius nearly ready to hatch ; side view ; *ant.*[1], antennule ; *ant.*[2], antenna ; *mn.*, mandible ; *ta.*, tail ; *c.sp.*, caudal spine ; *npl.eye*, Nauplius eye (black); *yk.end.*, yolk-endoderm.   At the side of the intestine and hind end of the stomach are seen flat granular cells.

CHTHAMALUS STELLATUS.   (Figs. 83–96.   × 360.)

Stage A.

Fig. 83. Ovum of *Chthamalus stellatus* in which the protoplasm and yolk are segregating.   *pv.*[3], second polar body.

* The colours in figs. 79–82 should be as in figs. 61–78.

Fig. 84. Ovum in which segregation of the protoplasm and yolk is complete, the boundary being transverse. The nucleus has appeared as a small clear spot in the protoplasm, just anterior to the basal plane ; around this the protoplasm, preparatory to the division of the nucleus, has assumed a radial arrangement ; $pv.^2$, second polar body.

Fig. 85. Example of an egg in which the basal plane was oblique.

Stage B.

Fig. 86. Example of an egg in which the second blastomere is arising before the separation of the first from the yolk along the basal plane has taken place.

Fig. 87. Egg showing the second blastomere arising from the yolk ; the first having been cut off by an oblique basal plane.

Fig. 88. Stage showing second blastomere arising from the yolk while the first is dividing.

Fig. 89. Stage with three blastomeres, and a fourth arising from the yolk on the left-hand side of II. (the cell on the left side of the figure).

Fig. 90. Stage with three blastomeres, and a fourth arising from the yolk in the middle line.

Fig. 91. Stage with five or six blastomeres.

Fig. 92. Stage in which the yolk is half covered by the blastoderm ; a new blastomere is emerging on the right-hand side of the yolk.

Fig. 93. Similar stage to that in fig. 92, but the merocyte in connection with the nearest blastomere by a nuclear spindle, seen by focussing below the surface.

Fig. 94. Stage in which the blastopore is being closed by the emergence of a merocyte.

Stage C.

Fig. 95. Stage in which the yolk (meso-hypoblast) is divided in two, each half being a cell with a nucleus and protoplasmic mass at its hinder end.

Fig. 96. Example in which the yolk had divided before the closure of the blastopore.

PLATE 19.

CHTHAMALUS STELLATUS.

Fig. 97. Stage showing commencement of the formation of the mesoblast of the Nauplius ; the yolk has lost its definite contour in the region where this

is taking place, and the mesoblastic cells themselves form a rather opaque mass (on the right-hand side of the figure).   × 360.

Stage F.

Fig. 98. Dorsal view of embryo-Nauplius with rather short appendages, taken out of the egg-shell ; *ant.*[1], antennule ; *ant.*[2], antenna ; *mn.*, mandible. The yolk endoderm is shaded.   × 360.

Stage H.

Fig. 99. Nauplius almost ready to hatch ; *ant.*[1], antennule ; *ant.*[2], antenna ; *mn.*, mandible ; *npl.eye*, Nauplius eye ; *lbr.*, labrum ; *ta.*, tail ; *c.sp.*, caudal spine.

LEPAS ANATIFERA.   (Figs. 101–113.   × 420.)

Stage A.

Fig. 100. Longitudinal section through ovum of *Lepas anatifera*, showing the nucleus dividing to form the second polar body ; *pv.*[1], first polar body ; the oil globules appear as clear round spaces.

Fig. 101. Oblique section of ovum in which the protoplasm and yolk are segregated, showing division of the segmentation-nucleus.   This stage would be a little later than that shown in fig. 7.

Fig. 102. Oblique section passing through stage between those shown in figs. 8 and 9.   The first blastomere has quite recently been cut off, and the spindle-fibres are still visible.

Fig. 103. Similar section of an egg in which the basal plane was oblique.

Fig. 104. A more longitudinal section of a rather later stage in which the two nuclei are completely disconnected ; *cf.* stage shown in woodcut *3f* (p. 196).

Fig. 105. Longitudinal section of an egg at the stage shown in woodcut *3g* ; *cf.* also figs. 9–11.

Stage B.

Fig. 106. Oblique section of an egg at stage a little later than that shown in fig. 17 ; *cf.* also fig. 17A ; a nucleus has recently been given off into the yolk from the third blastomere with which it is still in connection.

Stage C.

Figs. 107 and 108. Oblique sections passing through the two yolk cells (meso-
hypoblast) which have each cut off a mesoblastic cell ($mes.^1$); *cf.* wood-
cut 3*q* (p. 197).

Fig. 109. Sections of slightly later stage, in which the mesoblast cell has divided.

Fig. 109*a*. Next one in the series of sections of the same embryo.

Fig. 110. Section of a still later stage in which the yolk-cells have cut off two more
mesoblastic cells.

Fig. 111. Longitudinal section, showing yolk-endoderm divided into two ; mesoblast
cells behind. Lettering as in fig. 112.

Fig. 112. Longitudinal horizontal section of embryo at stage shown in fig. 28 ; several
yolk-endoderm cells present (four intersected). *Ect.*, ectoderm ; *mes.*[1],
mesoderm ; *yk. end.*, yolk-endoderm cell ; *yk. end. n.*, nucleus of yolk-
endoderm cell.

Stage D.

Fig. 113. Longitudinal vertical nearly median section of embryo at stage shown in
figs. 29–31, in which the body is apparently segmented into three por-
tions ; the section shows the dorsal mesoblastic plate ($mes.^1$).

PLATE 20.

LEPAS ANATIFERA.   (Figs. 114–121*f.*   × 420 ; figs. 122*a*–122*e.*   × 375.)

Fig. 114. Transverse section of same stage as that shown in fig. 113.

Stage E.

Fig. 115. Transverse section through an embryo with short appendages (figs. 35 and
36), taken between two pairs of appendages.

Fig. 116. Transverse section of same stage, taken through a pair of appendages.

Stage F.

Fig. 117. Longitudinal horizontal section through an embryo of Stage F (fig. 37) ;
the œsophagus (*stom.*) is cut transversely ; the intestine (*proct.*), longi-
tudinally ; *app.*, appendages ; *ta.*, tail.

Fig. 118. Transverse section through anterior region of embryo of same stage showing
the thin dorsal ectoderm, no longer covered by the free ends of the

antennules, the lower parts of which, with their mesoblastic tissue, are still closely adpressed to and not to be distinguished from the sides of the carapace ; ventral to the œsophagus is the tissue of the base of the labrum not clearly marked off in this section.

Fig. 119. Transverse section of embryo at same stage, taken through the *labrum* (*lbr.*) ; *app.*, appendage.

Fig. 120. Transverse section of embryo at same stage, taken through the intestine (*proct.*) ; *ect.*, ectoderm ; *app.*, appendage.

Stage G.

Fig. 121. Nearly median longitudinal vertical section of embryo-Nauplius with long appendages. *b.c.*, body cavity ; *br.*, brain ; *c.sp.*, caudal spine ; *ect.*, ectoderm ; *proct.*, intestine ; *sub.œs.g.*, sub-œsophageal ganglion ; *ta.*, tail ; *yk.end.*, yolk-endoderm ; *yk.end.n.*, yolk-endoderm nucleus. The boundaries between the individual yolk-endoderm cells are not clearly seen in this section.

Fig. 121*a*. Transverse section through embryo of same stage, taken anteriorly to the point at which the caudal spine separates from the tail (about the level of letters *ect.* in fig. 121). On each side of the tail are seen the exopodite (dorsal), and the endopodite (ventral) of the mandibles ; outside these come the exopodite (dorsal) and the endopodite (ventral) of the antennæ ; *b.c.*, body cavity.

Fig. 121*b*. Transverse section of embryo of same stage, taken some distance in front of the mouth. *lbr.*, labrum ; *œs.*, œsophagus ; *œs.c.m.*, circular muscles of œsophagus ; *yk.end.*, yolk-endoderm ; *yk.end.n.*, nuclei of yolk-endoderm cells.

Fig. 121*c*. Transverse section of same embryo, taken just anterior to mouth. Letters as in figs. 121*b*. *b.c.*, body cavity ; *c.o.c.*, circum-œsophageal connectives.

Fig. 121*d*. Transverse section of embryo of same stage, taken behind the mouth. Letters as in figs. 121*b* and 121*c*. *sub-œs.g.*, sub-œsophageal ganglion.

Fig. 121*e*. Half of a transverse section of embryo of same stage, passing through the fronto-lateral glands (*frl.gl.*). *frl.gl.n.*, nuclei of fronto-lateral glands.

Fig. 121*f*. Portions of longitudinal horizontal sections of embryo of same stage.

Fig. 122*a–e*. Sections showing the excavation of the yolk-endoderm to form the stomach, and the union of the œsophagus (*stom.*) and intestine (*proct.*) with the stomach. The boundaries of the yolk-endoderm cells are clearly seen ; the nuclei appear deeply stained. Figs. 122*a* and 122*d* pass through the stomach and œsophagus ; fig. 122*d* through the stomach and intestine ; and figs 122*b* and 122*e* through the stomach only.

BALANUS PERFORATUS.

Stage B.

Fig. 123. Nearly longitudinal section through an egg of *Balanus perforatus,* in which
the first blastomere has been cut off from the yolk, and the second is
forming (*mer.*).   × 420.

PLATE 21.

BALANUS PERFORATUS.   (Figs. 125–136*d*.   × 420.)

Stage B.

Figs. 124*a* and *b.* Longitudinal sections in planes at right angles to one another,
showing the first blastomere dividing transversely, and the second giving
off a nucleus into the yolk.   × 375.

Fig. 125. Nearly longitudinal section through an egg, with three blastomeres and a
merocyte (*mer.*) in the yolk.

Fig. 126. Longitudinal section through an egg, with six blastomeres, and a seventh
arising from the yolk.

Fig. 127. Section through a stage in which the blastopore is just being closed by a
merocyte (*mer.*).

Stage C.

Fig. 128. Longitudinal section of an embryo in which the blastoderm is completed;
a single merocyte, with its nucleus (*mer.n.*), is seen in the yolk.

Fig. 129. Oblique section of a stage in which the yolk (*meso-hypoblast*) has just
divided.

Fig. 130. Oblique section of stage with two yolk-cells (*meso-hypoblast*).

Fig. 131. Obliquely longitudinal section of stage, with about five or six endoderm
cells.   *mes.*[1], mesoblast.

Fig. 132. Transverse section, near hind end, of embryo at same stage.   *mes.*[1], meso-
blast.

Stage E.

Fig. 133. Transverse section of embryo with short appendages, before the appearance
of the œsophagus, showing mid-dorsal groove.

Stage F.

Fig. 134. Transverse section through an embryo-Nauplius at Stage F, taken through
the œsophagus (*stom.*). The labrum (*lbr.*) is seen as a low ventral pro-
jection ; *yk.end.n.*, nuclei of yolk-endoderm cells.

Fig. 135. Transverse section of the same embryo passing through the intestine (*proct.*), and appendages (*app.*).

Nauplius. Stage II.

Fig. 136*a*. Longitudinal section (considerably inclined to the sagittal plane) of Nauplius after the first moult, passing to one side of the labrum, mouth, and œsophagus. The communication between the stomach (*st.*) and intestine (*int.*) is well seen. The section also passes close to the anus. The "ventral plate" is cut parallel to its surface, so that its composition out of a single layer of cells is not clearly seen.

Fig. 136*b*. Longitudinal vertical section through posterior half of a Nauplius of Stage II., traversing stomach (*st.*), intestine (*int.*), sub-œsophageal ganglion (*sub-œs.g.*), and "ventral plate" (*ta.th.*).

Fig. 136*c*. Nearly transverse section, through Nauplius of same stage, passing through stomach (*st.*), œsophagus (*œs.*), circum-œsophageal connectives (*c.o.c.*), and labrum (*lbr.*). The œsophagus is cut through as it bends back dorsally and ventrally.

Fig. 136*d*. Nearly transverse section of Nauplius at same stage, passing through stomach (*st.*), mouth (*mo.*, on one side), labrum (*lbr.*), circum-œsophageal connectives (*c.o.c.*).

PLATE 22.

BALANUS PERFORATUS.

Nauplius. Stage II. (Figs. 137*a*–139*b*. × 420.)

Fig. 137*a*–*d*. Sections of Nauplius taken parallel to the labrum. *Ant.*[1], antennule; *ant.*[2], antenna; *app.d.m.*, dorsal muscles of appendages; *app.v.m.*, ventral muscles of appendages; *br.*, brain; *br.acc.l.*, accessory lobes of brain; *br.c.l.*, central lobe of brain; *br.p.l.*, posterior lobes of brain; *fr.fil.bs.*, base of frontal filament; *mnd.*, mandible; *npl.eye.*, Nauplius eye; *œs.*, œsophagus; *sub.œs.g.*, sub-œsophageal ganglion; *st.*, stomach.

Fig. 137*a*. Section passing immediately above mouth.

Fig. 137*b*. Section next but one higher.

Fig. 137*c*. Next section higher.

Fig. 137*d*. Next section higher.

Fig. 138. Horizontal section through anterior part of Nauplius, traversing brain, Nauplius eye, frontal filaments, stomach, and fronto-lateral glands. Letters as in figs. 137*a*–*d*.

Fig. 138*a*. Muscles as seen in section of same Nauplius.

Fig. 139*a*. Tranverse section of Nauplius taken in front of mouth, and just behind

the U-shaped bend of the œsophagus. *ax.gl.*, axial gland of labrum ; *ax.gl.n.*, nucleus of axial gland ; *car.*, edge of carapace ; *c.o.c.*, circum-œsophageal connectives ; *gn.*, gnathobase of antenna ; *gn.m.*, muscle of gnathobase ; *œs.*, œsophagus ; *st.*, stomach.

Fig. 139*b*. Transverse section of same Nauplius passing through the intestine (*int.*) and " ventral plate " (*ta.th*) ; *car.*, carapace.

Fig. 139*c*. Next section but one behind 139*b*.

Stage I.

Fig. 140. Dorsal view of Nauplius after moulting once. *·frl.h.*, fronto-lateral horns ; *gr.*, granular matter ; other letters as in figs. 137 and 139. × 220.

PLATE 23.

BALANUS PERFORATUS.

Stage II.

Fig. 141. Dorsal view of Nauplius after moulting once. *an.dil.*, dilator of anus ; *br.*, brain ; *br.acc.l.*, accessory lobes of brain ; *ect.*, ectoderm ; *fl.ta.*, flexor of the tail ; *fr.fil.*, frontal filament ; *fr.fil.bs.*, base of frontal filament ; *frl.gl.*, fronto-lateral gland ; *frl.gl.s.*, secretion of fronto-lateral glands ; *frl.h.*, fronto-lateral horn ; *int.*, intestine with its circular muscles ; *lat.gl.*, lateral gland ; *ves.t.*, vesicular tissue. × 220.

Fig. 142. Ventral view of Nauplius of same stage. *ax.gl.*, axial gland of labrum ; *c.sp.*, caudal spine ; *œs.*, œsophagus ; *gn.*, gnathobase of antenna ; *gn.m.*, muscle to ditto ; *ta.*, tail ; *ta.sp.*, tail spines : other letters as in fig. 141. On the ventral surface is seen the " ventral plate " and a number of rows of setæ. The longitudinal muscles of the gut are faintly seen through the setose region. × 220.

Stage I.

Fig. 143. Dorsal view of anterior half of a Nauplius of Stage I., in which are shown the brain, with the Nauplius-eye (*npl.eye*), and accessory lobes (*br.acc.l.*) ; the circum-œsophageal connectives (*c.o.c.*), and sub-œsophageal ganglion (*sub-œs.g.*) ; behind the eye is seen the " dorsal body " (*d.b.*), and behind this is seen the outline of the stomach (*arch.*) some of the cells of which are drawn : the optical section of the vertical part of the œsophagus is seen as a faint circle in the midst of these.

PLATE 24.

BALANUS PERFORATUS.

Nauplius, Stage II.

Fig. 144. Ventral view of the setose region of a Nauplius of Stage II., showing the arrangement of the bands of setæ.

Fig. 145. Ventral view of labrum of a Nauplius of Stage II. × 420. *ax.gl.*, axial gland of labrum ; *ax.gl.fi.*, fibre running to ditto ; *br.*, brain ; *ect.*, ectoderm ; *fr.fil.*, frontal filament ; *fr.fil.bs.*, base of ditto ; *lbr.c.*, cells at side of labrum ; *npl.eye*, Nauplius eye.

Fig. 146. Ventral view of thoracic region of a Nauplius of Stage II. *arch.*, stomach; *fl.ta.*, flexor of tail ; *gt.l.m.*, longitudinal muscles of stomach and intestine ; *ta.sp.*, tail-spines; *ta.th.*, ectodermal thickening of tail, or " ventral plate."

Fig. 147. Similar view showing a more advanced condition of the ventral plate. × 420.

Fig. 148. Portion of carapace of Nauplius (Stage II.), showing excreted granules of methyl-blue. × 420.

Fig. 148*a*. Labrum of Nauplius (Stage II.), showing excreted granules of methyl blue. × 420.

CHTHAMALUS STELLATUS.

Fig. 149. Dorsal view of Nauplius (Stage II.) of *Chthamalus stellatus*. *app. d. musc.*, dorsal muscles of appendages ; *br.*, brain ; *br.acc.l.*, accessory lobes of brain ; *int.*, intestine ; *fr.fil.bs.*, base of frontal filaments ; *frl.gl.*, fronto-lateral gland ; *frl.gl.s.*, secreted spherules of ditto ; *frl.h.*, fronto-lateral horns; *lat.gl.*, lateral gland ; *st.*, stomach ; *ves.t.*, vesicular tissue. The sub-cuticular network is shown on the right-hand side, and the deeper structures only on the left. × 212.

PLATE 25.

CHTHAMALUS STELLATUS.

Nauplius, Stage II.

Fig. 150. Ventral view of Nauplius (Stage II.) of *Chthamalus stellatus*. *ax.gl.*, axial gland of labrum ; *br.*, brain, with Nauplius eye resting upon it ; *c.sp.*, caudal spine ; *fr.fil.* frontal filament ; *lat.gl.*, lateral gland ; *lbr.dist.*

distal lobe of labrum ; *lbr.prox.*, proximal lobe of ditto ; *ta.sp.*, tail-spine. × 216.

Fig. 151. Lateral view of Nauplius (Stage II.) of *Chthamalus stellatus. anus ; an. arc*, anal arc ; *app.d.musc.*, dorsal muscles of appendages ; *lbr.*, labrum ; *st.*, stomach ; *ta.*, tail ; other letters as in fig. 150.   × 220.

Fig. 152. View of portion of ventral surface of Nauplius (Stage II.) of *Chthamalus stellatus. an.arc*, anal arc ; *c.sp.*, caudal spine ; *ex.mx.arc*, extra-maxillary arc ; *fl.arc*, flexor arc ; *fl.ta.*, flexor of tail ; *lbr.*, labrum ; *pmx.bd.*, pre-maxillary band ; *ta.*, tail ; *ta.sp.*, tail-spine.   × 265.

LEPAS ANATIFERA.

Stage I.

Fig. 153. Ventral view of a Nauplius of *Lepas anatifera* just hatched, showing *brain* (*br.*), with the *frontal filaments* (*fr.fil.*) underneath the cuticle, and their bases (*fr.fil.bs.*) in the brain on each side of the Nauplius eye ; the labrum (*lbr.dist.* and *lbr.prox.*) ; the fronto-lateral horns (*frl.h.*) are bent back parallel to the body, and the caudal spine and tail telescoped within the body, their tips only being as yet external ; portions of the fronto-lateral glands are seen just in front of the antennules, and a portion of the setose region behind the labrum.   × 220.

Fig. 153a. Dorsal view of same. *app.d.musc.*, dorsal muscles of appendages ; *arch.*, stomach ; *c.sp.*, caudal spine ; *fl.ta.*, flexor of tail ; *frl.gl.*, fronto-lateral gland ; *gr.mat.*, granular matter ; above the Nauplius eye is seen the " dorsal body."   × 220.

Stage II.

Fig. 154. Nauplius of *Lepas anatifera* just after the first moult, with the labrum turned a little forwards, so as to expose the mouth (*mo.*), and the origin of the ventral muscles running to the appendages (*app.v.musc.*), as well as the sub-œsophageal ganglion (*sub.œs.g.*). The tail and caudal spine are still telescoped within the body.   × 140.

Fig. 155. Ventral view of a more advanced Nauplius of Stage II., not long after the first moult, with the labrum (*lbr.*) turned forwards. The tail and caudal spine are still telescoped within the body.   × 220.

PLATE 26.

LEPAS ANATIFERA.

Stage II.

Fig. 156. Dorsal view of a fully-developed Nauplius (Stage II.) of *Lepas anatifera.*
an.dil., dilator of anus; *app.d.musc.,* dorsal muscles of appendages; *br.,*
brain; *d.b.,* dorsal body; *fl.ta.,* flexor of tail; *frl.h.,* fronto-lateral horn;
frl.gl., fronto-lateral gland; *frl.gl.n.,* nuclei of ditto; *st.,* stomach, fol-
lowed by intestine with its circular muscles; *st.b.,* stellate body; *ves.t.,*
vesicular tissue; in front of the anterior margin are seen the frontal
filaments. × 220.

PLATE 27.

LEPAS ANATIFERA.

Nauplius. Stage II.

Fig. 157. Ventral view of a fully-developed Nauplius after moulting *once* (Stage II.).
an.arc, anal arc; *ant.arc,* anterior arc; *ax.gl.,* axial gland of labrum;
c.sp., caudal spine; *d.b.,* dorsal body; *exm.arc,* extra-maxillary arc;
fl.arc, flexor arc; *frl.gl.,* fronto-lateral gland; *frl.gl.s.,* secreted spherules
of ditto; *lbr.,* labrum; *œs,* œsophagus; *ta.,* tail; *ta.sp.,* tail-spine. × 220.

Fig. 158. Ventral view of labrum and part of nervous system of a Nauplius of the
same stage. *Ax.gl.,* axial gland of labrum; *br.,* brain; *c.o.c.,* circum-
œsophageal connectives; *lbr.c.,* cells at sides of labrum; *lbr.dist.,* distal
lobe of labrum; *lbr.prox.,* proximal lobe of labrum; *œs.,* œsophagus with
its circular muscles. × 420.

Fig. 159. View of labrum of Nauplius of same stage turned forwards to expose the
mouth and hinder part of the nervous system. *Mo.,* mouth; *c.o.c,* circum-
œsophageal connectives; *sub-œs.g.,* sub-œsophageal ganglion: other letters
as in fig. 158.

Fig. 160. View from below of setose region of Nauplius of same stage. *Fl.ta.,* flexor
of tail; *prmx.bd.,* premaxillary band; *ta.th.,* initial stage of ventral
plate: other letters as in fig. 157.

· LEPAS PECTINATA.

Stage I.

Fig. 161. Nauplius of *Lepas pectinata* just hatched. *App.d.m.,* dorsal muscles to
appendages; *br.,* brain; *frl.gl.,* fronto-lateral glands at base of fronto-
lateral horns; *gr.mat.,* granular matter; *int.,* intestine; *st.,* stomach.
× 220.

PLATE 28.

LEPAS PECTINATA.

Nauplius. Stage II.

Fig. 163. Ventral view of setose region of Nauplius (Stage II.) of *Lepas pectinata.*
　　　　× 420.

CONCHODERMA VIRGATA.

Nauplius. Stage I.

Fig. 164. Dorsal view of Nauplius of *Conchoderma virgata* just hatched ; *app.d.m.*,
　　　　dorsal muscles of appendages ; *br.*, brain, with Nauplius eye ; *c.sp.*, caudal
　　　　spine, telescoped within the body, together with the tail ; *frl.h.*, fronto-
　　　　lateral horn ; *frl.gl.*, fronto-lateral gland ; *gr.mat.*, granular matter ; *st.*,
　　　　stomach. × 220.

Fig. 165. Dorsal view of Nauplius of *Conchoderma virgata* some time after the first
　　　　moult. The tail, caudal spine, and setæ are beginning to evaginate ;
　　　　fl.ta., flexor of tail ; *frl.fil.*, frontal filaments ; *frl.gl.s.*, secretion of
　　　　fronto-lateral gland ; *ta.*, tail ; other letters as in fig. 164. × 220.

Fig. 166. Ventral view of more advanced condition of Nauplius of same Stage (II.),
　　　　showing the further evagination of the tail and caudal spine ; *br.*, brain ;
　　　　c.sp., caudal spine ; *lbr.dist.*, distal lobe of labrum ; *lbr.prox.*, proximal
　　　　lobe of labrum ; *œs.*, œsophagus ; *ta.*, tail. × 220.

DICHELASPIS DARWINII.

Nauplius. Stage I.

Fig. 167. Dorsal view of Nauplius of *Dichelaspis Darwinii* in the first stage ; *arch.*,
　　　　stomach ; *ax.gl.*, axial gland of labrum ; *br.*, brain ; *fl.ta.*, flexor of tail ;
　　　　lbr.dist., distal lobe of labrum ; *lbr. prox.*, proximal lobe of labrum ;
　　　　npl.eye, Nauplius eye ; *œs.*, œsophagus. The tail and caudal spine are
　　　　telescoped within the body, and the fronto-lateral horns are seen lying
　　　　parallel to the sides of the body. × 220.

Fig. 168. Ventral view of a Nauplius of *Dichelaspis Darwinii* at Stage II, as made,
　　　　out from a number of mounted imperfect specimens ; *ax.gl.*, axial gland of
　　　　labrum ; *c.sp.*, caudal spine ; *fl.ta.*, flexor of tail ; *frl.fil.*, frontal filaments ;
　　　　frl.h., fronto-lateral horn ; *npl.eye*, Nauplius eye ; *ta.*, tail ; *ta.th.*, rudiment
　　　　of ventral plate. × 110.
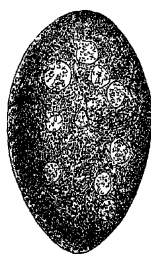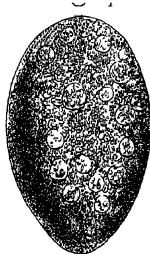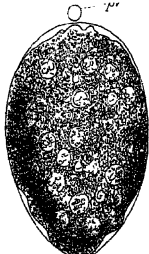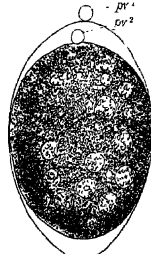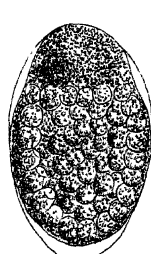
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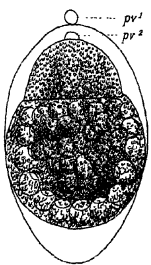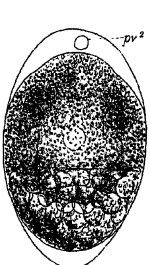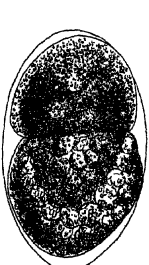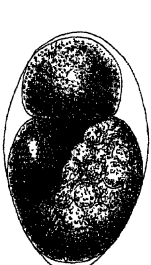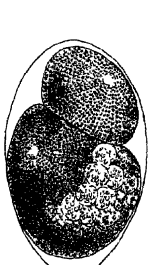
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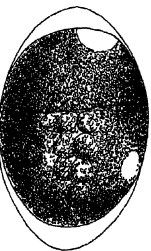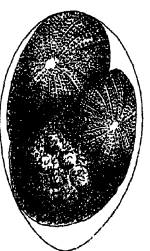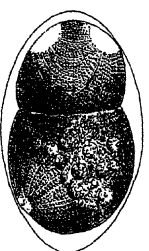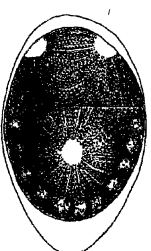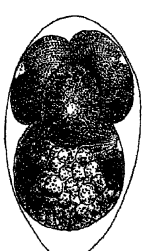
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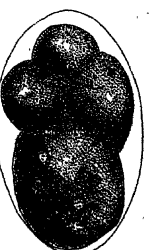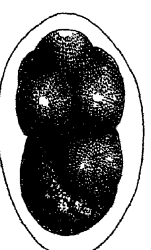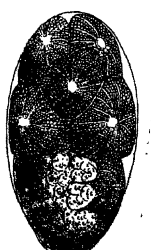
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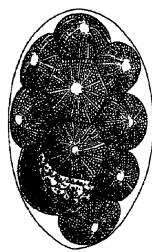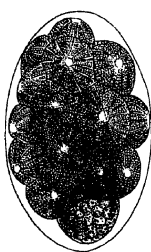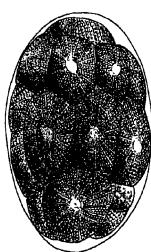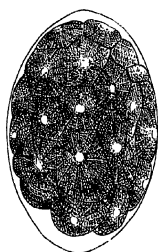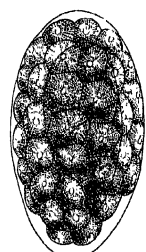
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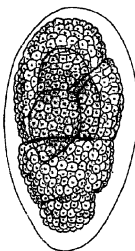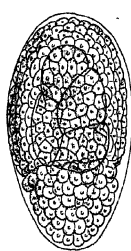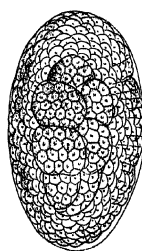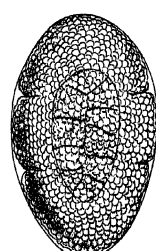
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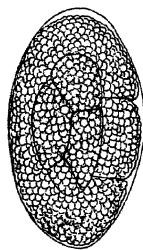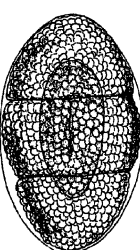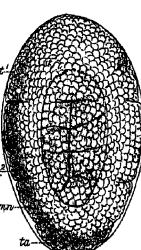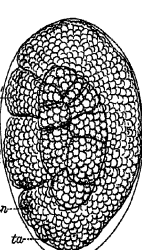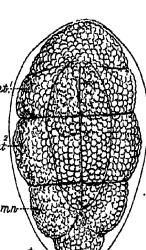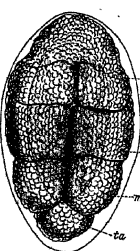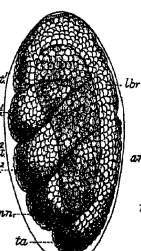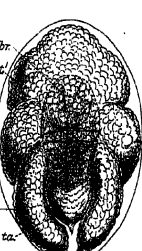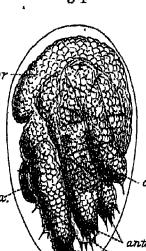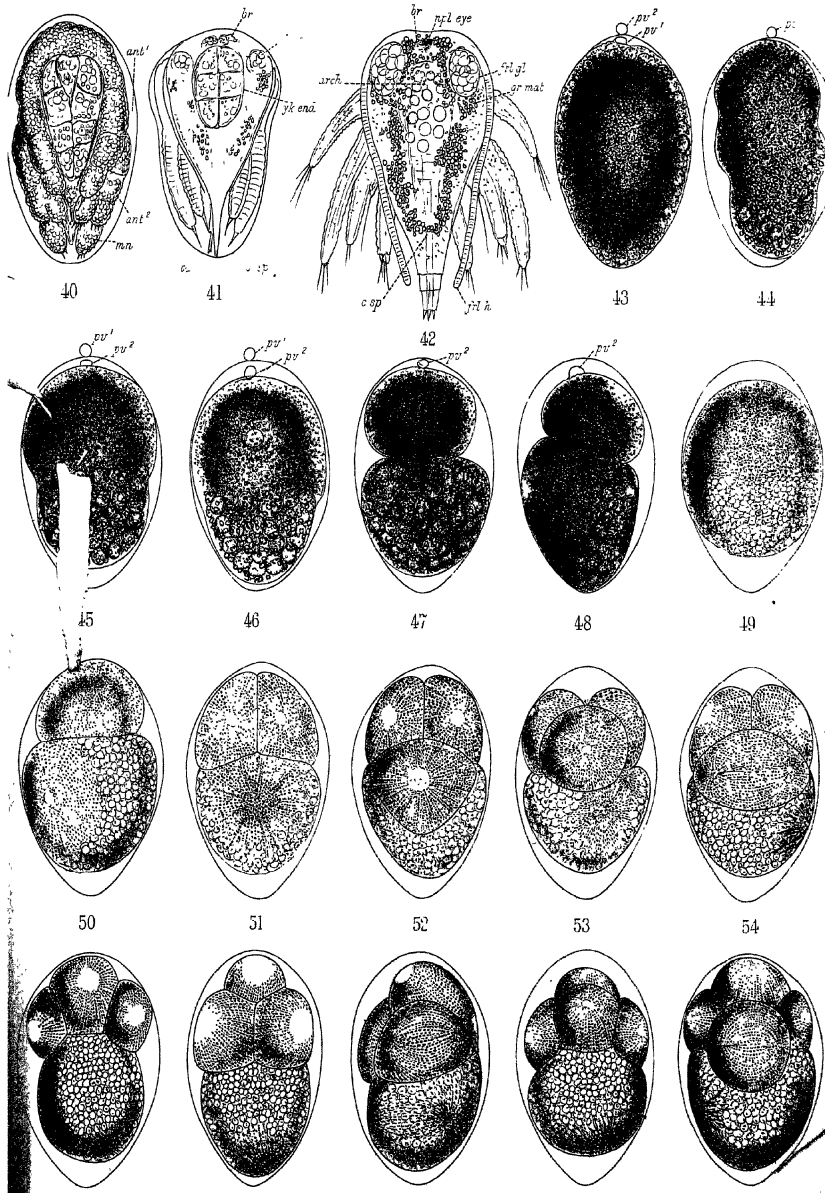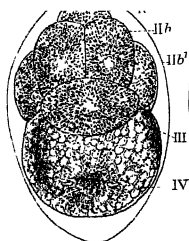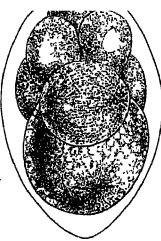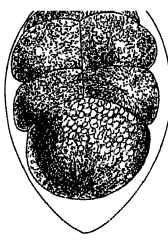
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79   80   81   82

83   84   85   86   87

88   89   90   91

Figure labels (as visible in illustrations):

98

99 — *yk.end.*, *npl eye*, *lbr*, *ant'*, *ant.'*, *ant²*, *mn*, *ant'²*, *mn*, *ta*, *c sp*

101

102

103

104

105

107

108 — *mes.*

109 — *mes'*

109 *a* — *mes.'*

yk.end c., *yk end n.*, *yk end n.*

yk end n., *yk.end*, *ect.*, *mes.'*

mes.', *mes.'*, *mes.'*, *mes'*, *yk.end*

114

115

116

117

118

119

120

121 a

121 b

121 c

121

122 a

121 e

121 f

122 d

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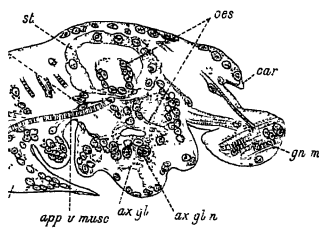
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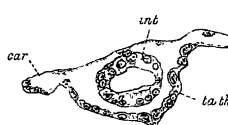
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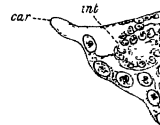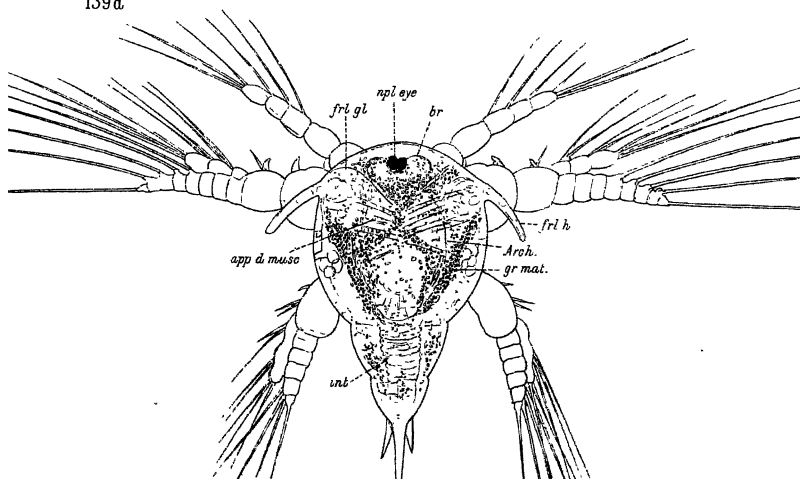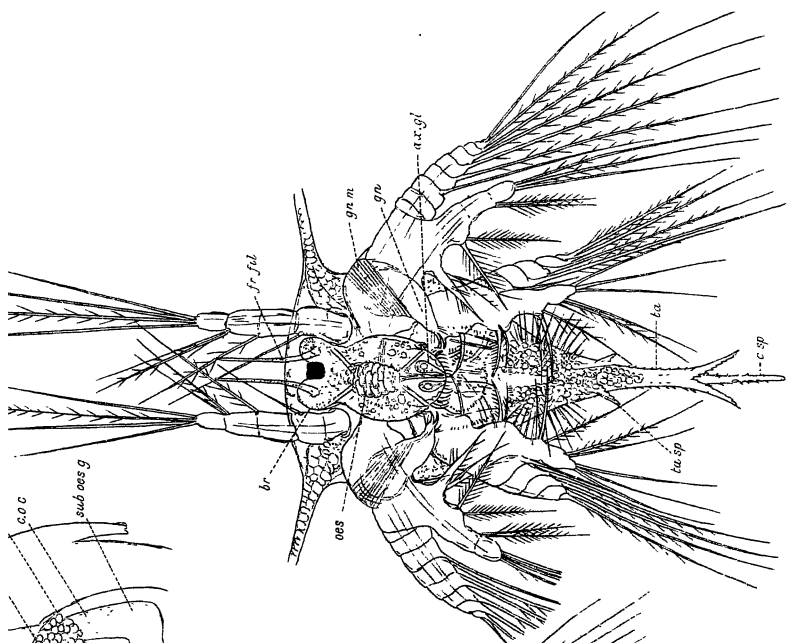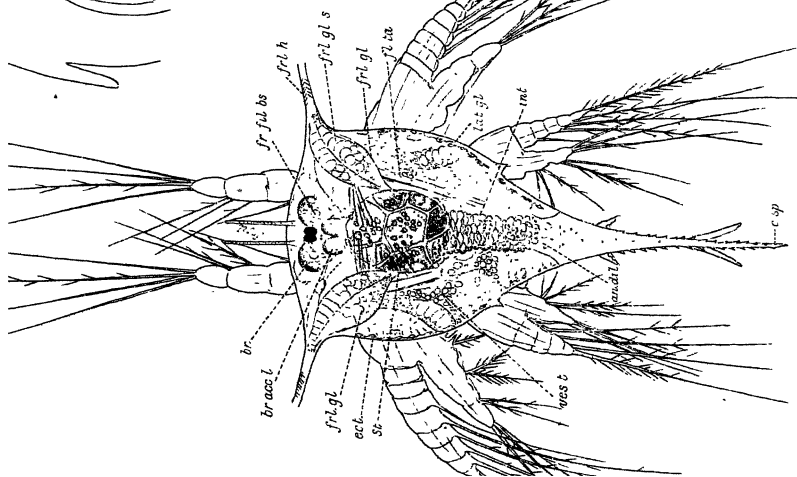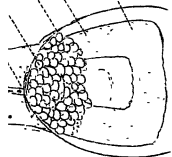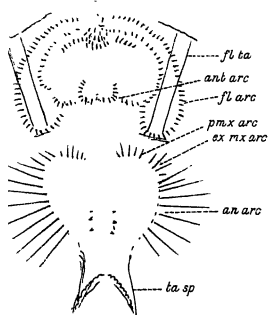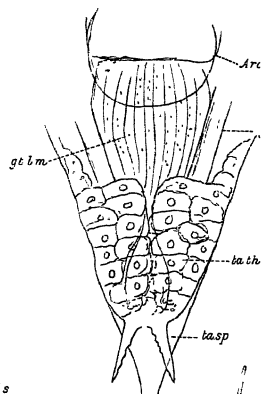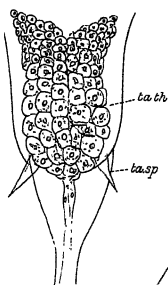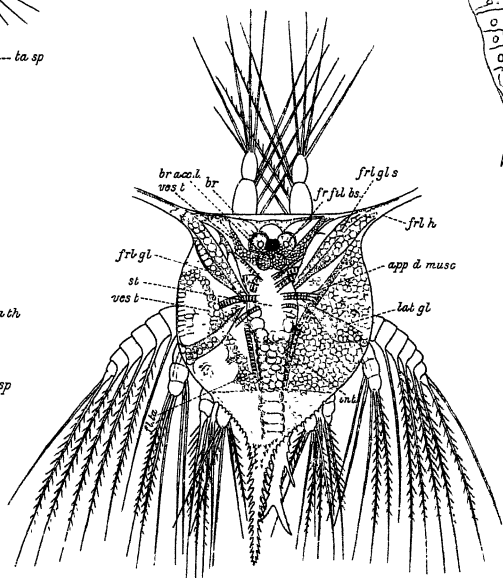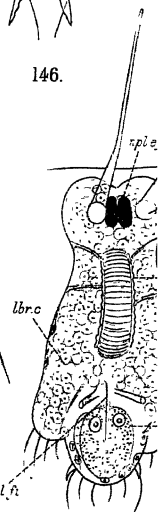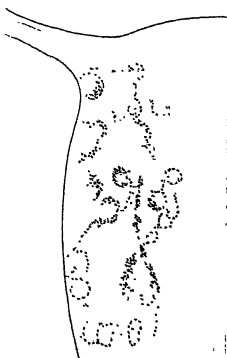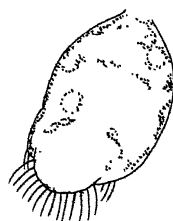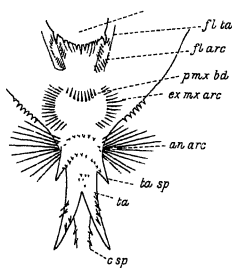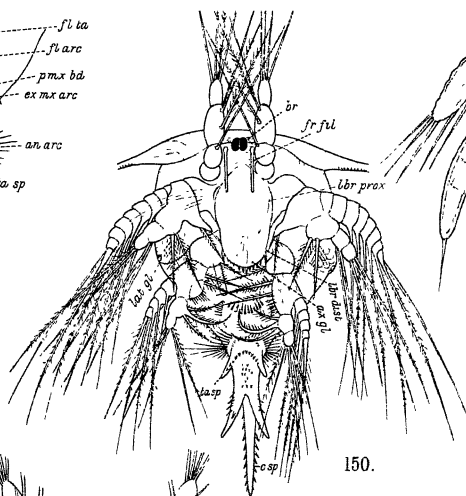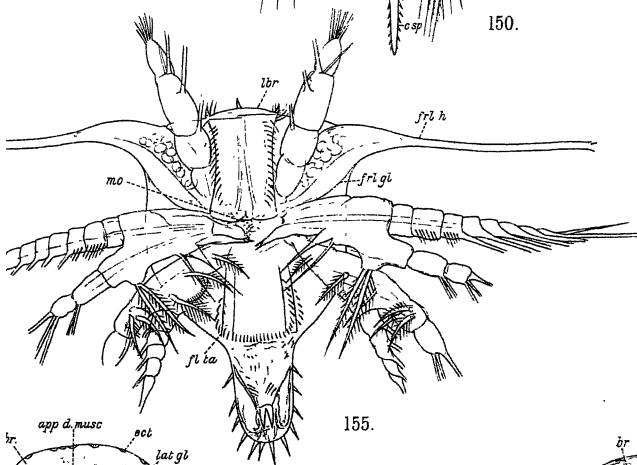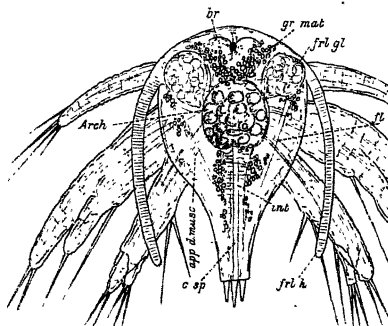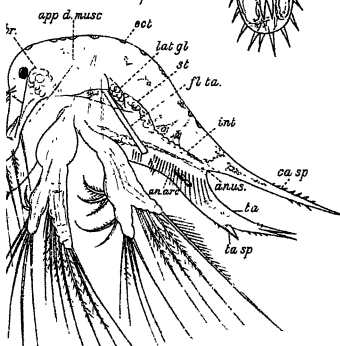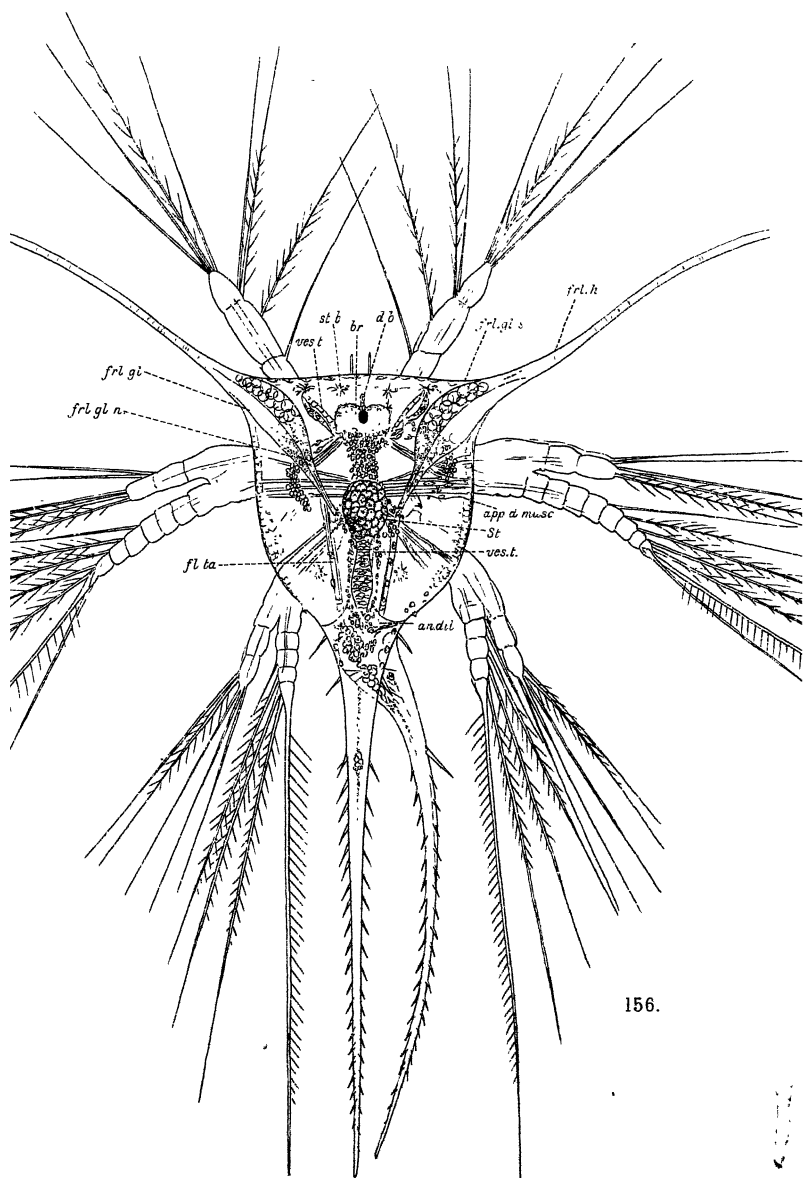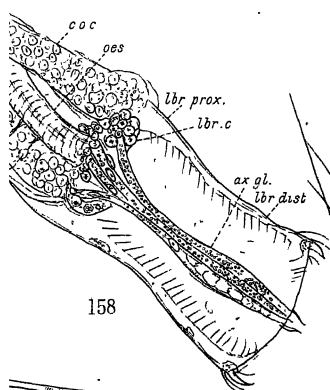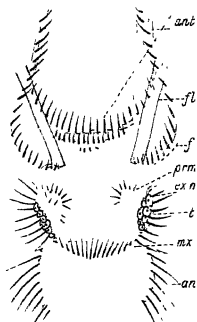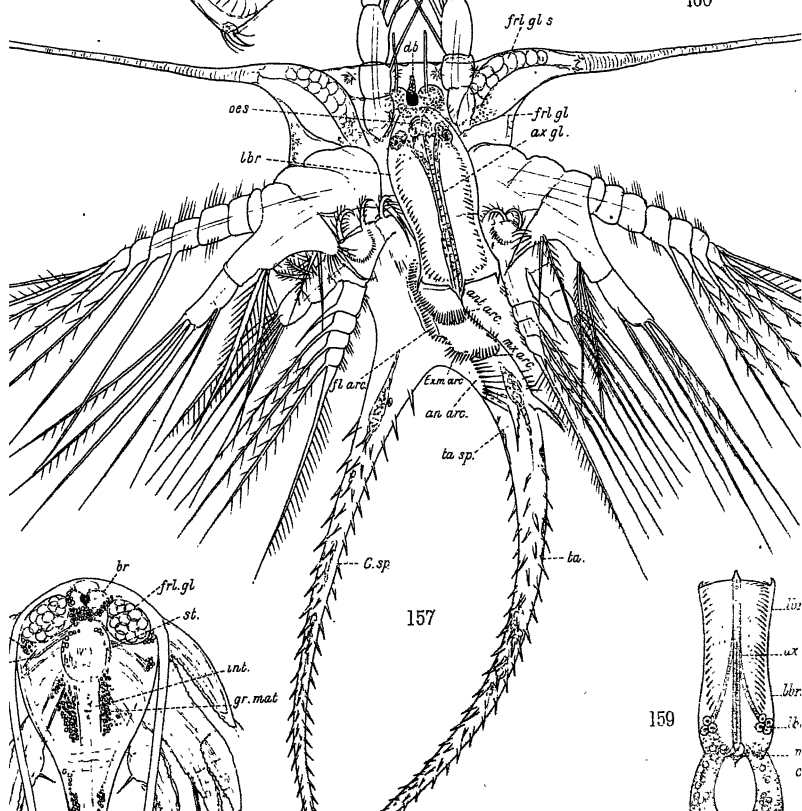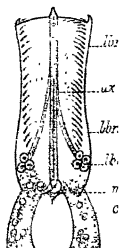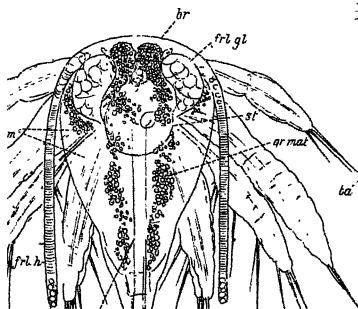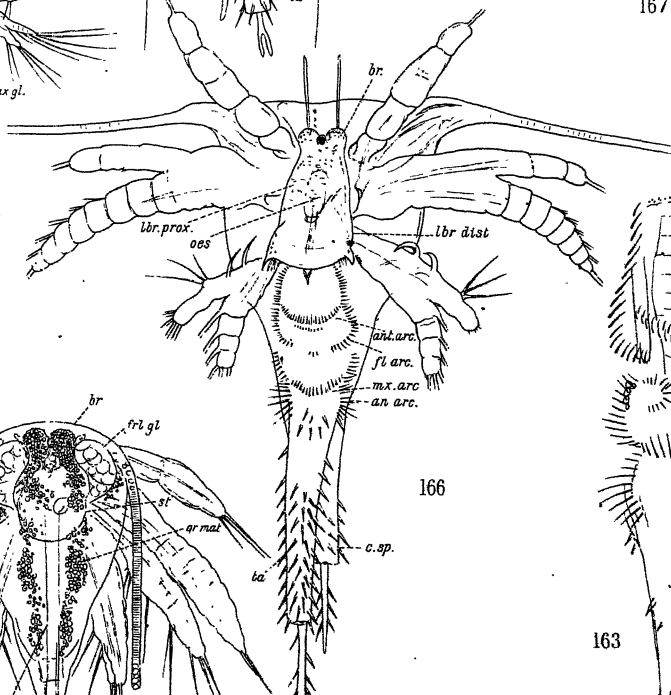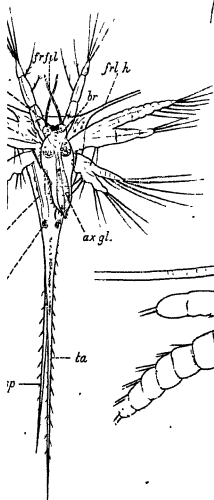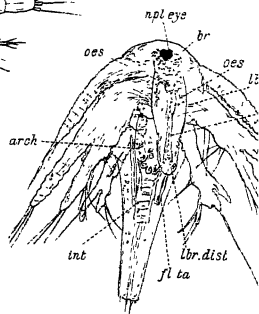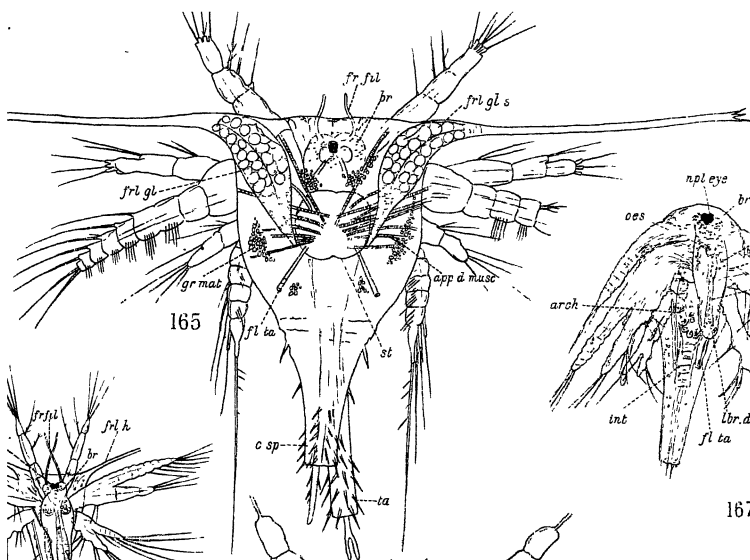
VI. *On Hepatic Glycogenesis.*

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PRELIMINARY.

ALTHOUGH hepatic glycogenesis was undiscovered before 1854, a large number of investigators of animal metabolism have directed their attention to its elucidation, and the literature on the subject has thus become very extensive. In spite of this, our knowledge of the physiology of the process is far from complete or satisfactory.

The term "glycogenesis" is here used in the sense in which it was originally employed by CLAUDE BERNARD, *i.e.*, the "production of sugar," and not the mere formation of glycogen—a sense in which it is too commonly employed by many writers.

That one of the great functions of the liver is to produce sugar will not, at the present time, be denied by any physiologist.*

The question, therefore, resolves itself into—

I. From what, and how, is hepatic sugar produced? And this at once leads to the question of—What is the relationship of hepatic sugar to glycogen?

BERNARD and the vast majority of subsequent investigators have been led to the conclusion that glycogen is the forerunner of the sugar. SEEGEN alone opposes this view, and bases his opposition on the following grounds† :—

1st. That, during starvation, sugar is produced in the liver up to the last hours of life, and long after all the glycogen has disappeared from the organ.

2nd. That, according to the researches of KRATSCHMER and himself, the production of sugar in the post-mortem liver is in excess of the disappearance of glycogen.

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* The theory of PAVY, repeated in nearly every text-book, that the liver is "a sugar destroying, and not a sugar forming organ," rests on so unsubstantial a basis and has been so completely refuted by the work of SEEGEN and other investigators that it need not be considered.

† PAVY's position, of course, necessitates the conception that hepatic glycogen has a fate other than ar.

To draw such a conclusion from the former fact shows a want of appreciation of the relationship of glycogen to the sugar, so clearly recognized by BERNARD. It is when a supply of glycogenic (*i.e.*, sugar yielding) material exists in the liver that it is stored as glycogen, just as in a resting gland cell, when there is no call for mucin, or the special zymin, an accumulation of mucinogen or of zymogen occurs. If the demand for sugar in the organism is urgent, the liver is deprived of the material for the formation of this substance. To argue the non-relationship of sugar to glycogen for this reason is as absurd as to argue the non-relationship of mucin and the zymins to the mucinogen and the zymogens, because, in the prolonged activity of the cells, these latter bodies are not accumulated and stored.

Against the experimental basis of the second objection must be set the observations of BOEHM and HOFFMANN ('Pflüger's Archiv,' vol. 23) and of GIRARD ('Pflüger's Archiv,' vol. 41), which clearly indicate that the amount of sugar formed in the liver post-mortem is proportionate to the glycogen disappearing.

But even supposing that SEEGEN and KRATSCHMER's results are correct, they by no means justify the conclusions founded upon them. The fact that glycogen is formed, not only from carbohydrates, but also from proteids, and the possibility that the vital functions of the cells of the body are continued for some time after the death of the animal, might explain the post-mortem production of the slightly increased amount of carbohydrates found by these investigators.*

With such conclusive evidence, further experiments are needless.

Glycogen being the forerunner of sugar, the problem of glycogenesis resolves itself into :—

1st. From what and how is glycogen formed in the liver ?

2nd. How is it related to the protoplasm of the liver cells ?

3rd. Into what kind of sugar is it changed, and how is the conversion brought about ?

As a result of his investigations, BERNARD ('Leçons sur la Diabète,' p. 307) gives as his answer to the first and third of these questions, that the process of sugar formation in the liver, " s'accomplit en deux actes. . . . L'acte *chimique*, c'est la transformation du glycogène en sucre. L'acte *vital*, c'est la production du glycogène au sein du tissue vivant."

All subsequent work has but confirmed the latter part of this statement, and has fully demonstrated that the progenitors of glycogen probably first become part of the living protoplasm of the cells before being changed into that substance. The literature on this aspect of the question is very extensive. Among the most important communications are those of VOIT and his scholars, the main results of which are given in vol. 28 of the 'Ztsch. f. Biol.' (" Ueber die Glykogenbildung nach Aufnahme verschiedener Zuckerarten"); while the whole evidence is ably considered and con-

* FRANKEL's theory of the mode of storage of glycogen in the cell ('Pflüger's Archiv,' vol. 52) may also afford an explanation of such results.

densed by Pflüger ("Ueber die Synthetischen Processe und die Bildungsart des Glykogens im thierischen Organismus."—'Pflüger's Arch.,' 1888).

The results of more recent observations have not, however, confirmed the former part of the statement, that the conversion of glycogen to sugar is a purely *chemical* act; and at present there is a tendency to view the conversion of glycogen as due, not merely to a chemical process, the result of the action of a soluble ferment or zymin, but to some process connected with the metabolic changes in the liver-cells.

While Bernard was undoubtedly influenced, in coming to his conclusion, by the then recent discovery of the amylolytic action of the *zymins*—diastase, ptyalin, and the pancreatic ferment—the physiologists of the present day, influenced by the discovery of the true nature of the activity of secreting epithelium, naturally tend to regard the processes in the liver-cells as identical with these.

The direct experimental evidence on this matter is, however, far from satisfactory; and the object of this paper is to attempt to give a definite answer to this important question.

In considering this question it is necessary to bear in mind the chemical relationships of the various carbohydrates.

The simplest members of the group formerly known as the glucoses, now better known as the monosaccharids, are the aldehyds (*e.g.*, dextrose) or ketones (*e.g.*, lævulose) of a hexatomic alcohol, occurring in at least three isomeric forms, having the formula $C_6H_8(OH)_6$.

By the polymerization of two molecules of such monosaccharids with dehydration, the group of disaccharids, having the formula $C_{12}H_{22}O_{11}$ is formed. Of this series, the members best known are maltose, formed by the union of two molecules of dextrose; saccharose, or cane sugar, formed by the union of a molecule of dextrose and of lævulose; and lactose, composed of a molecule of dextrose and one of galactose.

By further polymerization and dehydration a series of polysaccharids, consisting of the dextrins and starches, animal and vegetable, and the gums, is produced. The most complex have molecules of enormous size, the molecule of starch being possibly composed of no less than 120 dehydrated monosaccharid molecules, $120(C_6H_{12}O_6-H_2O)$. From such bodies to the disaccharids, the dextrins form a continuous chain of less and less complex substances.

The conversion of glycogen to sugar is thus a double one of disintegration and hydrolysis. It may be brought about by various agencies.

1st. By the action of dilute mineral acids at a high temperature.

2nd. By the action of such *zymins* as occur in the saliva and pancreatic juice.

3rd. By the action of various micro-organisms.

4th. By an unknown process in the liver during life and after death.

It is with the nature of this process that we have at present to deal.

The possibility of the process being due to the action of an acid in the *living* body may be at once dismissed. The post-mortem development of acid in the liver some-

what complicates the problem, and this part of the subject will be afterwards dealt with. The question is, therefore, whether the change is due to a zymin developed in the liver, or whether it is dependent on the metabolic processes which constitute the life of the liver cells.

That an amylolytic ferment can be extracted from the dead liver there can be no doubt. EVES ('Journ. of Phys.,' 1884, vol. 5) gives a *résumé* of the literature upon the existence of such a ferment, and points out that the evidence seems to show that "the liver can scarcely be regarded as a more prolific source of such ferment than are the other tissues of the body."

Her experiments conducted in the Physiological Laboratory of Cambridge showed that an amylolytic ferment may undoubtedly be isolated from the liver of the sheep, which brings about a slow and incomplete conversion of glycogen into a sugar, which she considered to be probably maltose. The nature of the sugar produced we shall again have to refer to.

DASTRE ('Arch. de Physiol.,' 1888, p. 76) also considers the literature on the isolation of an hepatic amylolytic ferment, and mentions the researches of EPSTEIN and MÜLLER, and of ABELES, which are not quoted by EVES. In his experiments he endeavours to show that the so-called ferment action is really due to the influence of micro-organisms, and not to a zymin. (For the experiments on which he bases this conclusion, see *loc. cit.*, p. 81, *et seq.*).

As to the *intra vitam* conversion of glycogen he says, "la transformation du glycogène en sucre n'est pas le résultat de l'intervention d'une diastase, séparable, isolable. Elle est le fait de l'activité vitale des cellules hépatiques."

To establish this thesis he endeavoured to show that all factors which slow or arrest the activity of cells, slow or arrest the transformation of glycogen; while they do not act in the same way on the activity of diastase (p. 94).

He points out that LANGENDORFF in 1886 ('Arch. de Physiol.') expressed the same view, without giving any evidence. A similar view is expressed, though also without experimental evidence, by EVES (*loc. cit.*), by RANSOME ('Journal of Physiol.,' 1887, p. 113), and by NEUMEISTER ('Lehrbuch der physiologischen Chemie,' 1893, p. 258). NEUMEISTER says, p. 259, "Die Leber, einem lebenden Tiere schnell entnommen und sogleich in siedendes Wasser verbracht, enthält in der That Zucker, dessen Menge 0.2–0.6 Proz. beträgt. Diese Zuckermenge vermehrt sich allerdings schnell beim Liegenlassen des ausgeschnittenen Organs, aber keineswegs durch einen postmortalen Vorgang, sondern im Gegenteil, weil das überlebende Protoplasma der Leberzellen noch weiter umsetzend auf das Glykogen einwirkt, während der gebildete Zucker nicht durch die Cirkulation fortgeführt wird."

While such observations go far to throw doubt upon BERNARD's conclusion that the conversion of glycogen to sugar is a chemical act—the result of the action of a soluble ferment, and independent of the life of the liver cells, they cannot be considered to disprove it. The recent researches of BIAL and ROHMANN ('Pflüger's Arch.,' vols. 52, 53

and 54) on the diastatic ferment of the blood, producing a true glucose from glycogen and starch, would rather favour the older view of BERNARD.

The question must still be considered as open and requiring further investigation.

In carrying out such investigation, the first question to be answered is—how far is the process of glycogen conversion dependent on, or independent of, the life of the liver cells?   The enormous post-mortem production of sugar from glycogen would at first sight seem to indicate that the process is independent of the vitality of the liver.   But the continued life of many tissues, e.g., of muscles, &c., after the death of the animal, must be borne in mind in considering the changes in the liver after somatic death.

The first part of this research then concerns :—

I. THE RELATIONSHIP OF GLYCOGEN CONVERSION TO THE CONDITION OF THE LIVER CELLS.

For this purpose the excised liver was used.   As the object of the experiment was not to maintain as long as possible the vital processes in the liver cells, but merely to *contrast their results* with the changes subsequently occurring, it was not considered necessary to maintain the circulation by the perfusion method.

The roughly-minced organ, from the animal just killed by a blow behind the head and then bled, was placed in 0·75 per cent. salt solution, and maintained at the temperature of the body in an incubator with or without a current of air.

This method of studying the chemical changes in various organs, with the use of diluted defibrinated blood instead of salt solution, was first used by PFLÜGER.   In a paper, " Der lebendige Organbrei und die Topographie des physiologischen Chemismus" ('Pflüger's Arch.,' vol. 23, p. 172), he strenuously defends its use against the attacks of ANDEER and W. KOCHS.

In the hands of BUNGE and SCHMIEDEBERG, and of SCHMIDT and his Dorpat scholars, the method has also yielded valuable results.

In endeavouring to trace the connection of glycogen conversion to the condition of the liver cells, it is necessary, in the first instance, to have a knowledge of the rate at which the glycogen disappears, at various periods, in the special conditions under which the liver is being observed.

A. THE RATE OF CONVERSION OF GLYCOGEN.

. This has not been investigated in the special conditions described above.   On the excised liver simply kept at the temperature of the room two series of observations have been made bearing upon this question.

SEEGEN ("Studien über Stoffwechsel," p. 405, *et seq.*), in a series of observations, in which he endeavoured to disprove the connection of sugar formation with glycogen formation, estimated the glycogen and glucose in the liver at different periods *post-mortem*,

He gives a table of the amount of glucose found in the liver of five dogs, which shows that in the first hour there was a gain of 43·5, 39·4, 40·0, 44·3, 67·7 per cent., an average of nearly 47 per cent. During the next twenty-four hours the gain was 28·7, 41·4, 26·0, 40·1, and 32·0 per cent., an average of 33·6 during the whole period, or of 1·4 per cent. per hour.

DALTON ('Treatise on Human Physiology,' p. 196) gives the results of three observations on the *post-mortem* production of sugar in the liver, from which the following table has been constructed.

EXPERIMENT 15.

Five seconds after death, liver contained 0·181 per cent. glucose. If none was present during life, during this interval glucose must have been produced at the rate of 1·372 per cent. per minute.

Fifteen minutes after death, the liver contained 0·679 per cent. glucose, an increase of 0·498 per cent., or of 0·033 per cent. per minute. Sixty minutes after death, the liver contained 1·026 per cent. glucose, an increase of 0·347 per cent., or of 0·007 per cent. per minute.

EXPERIMENT 19.

Five seconds after death, liver contained 0·3854 per cent. glucose, equivalent to the production of 4·248 per cent. per minute.

After six hours, the liver contained 1·1458 per cent., an increase of 0·7604, or of 0·0021 per cent. per minute.

EXPERIMENT 20.

Four seconds after death, the liver contained 0·2675 per cent. glucose, a production of 4·012 per cent. per minute. One hour after death there was 1·1888 per cent. glucose, an increase of 0·9213, equal to 0·015 per cent. per minute.

Four hours after, the glucose was 1·3361 per cent., an increase of 0·1475 per cent., or 0·0008 per cent. per minute. Twelve hours after, the glucose was 1·5317 per cent., an increase of 0·199 per cent., or 0·0004 per cent. per minute.

Both these series of experiments indicate an enormously active production of sugar in the liver just after death, and a progressively slower production during a later period.

From these observations it is impossible to draw absolutely definite conclusions as to the actual production of glucose or the disappearance of glycogen, because it is quite possible that carbohydrates other than glucose may be produced, and that the sugar, after being produced, may be destroyed. The observations of LÉPINE and BARRAL ('Comptes Rendus,' vol. 112 and 113) on the glycolytic action of the blood go far to prove the possibility of this occurring.

For this reason it was considered desirable to investigate directly the changes in hepatic glycogen at different periods after death, the liver being kept in the condition above described.

In these experiments, rabbits in a good state of nutrition, usually fed on oats and

bran with some green food, were used. The animal was killed by a blow behind the ears, and the carotids were at once severed, and the animal thus thoroughly bled. The abdomen was opened, and the inferior cava and portal vein were found collapsed and containing little blood. The liver was excised and the gall bladder torn away from it. On squeezing little blood could be expressed. The organ was then rapidly minced with a sharp razor and divided into several parts.

One of these was at once thrown into actively boiling water. The other portions were placed in wide-mouthed bottles containing about 150 cub. centims. of 0·75 per cent. salt solution, at a temperature of 37° to 40° C., and kept at this temperature in an incubator with or without a current of air for definite periods. At the end of the period the bottle was removed, and the contents thrown into boiling water and boiled actively for five minutes.

For the extraction of glycogen BRÜCKE's method was usually employed. In some cases KÜLZ's potash method was used. VINTSCHGAU and DIETL ('Pflüger's Arch.,' vol. 13, pp. 253, 187) have shown that boiling with caustic potash causes a disappearance of a considerable quantity, sometimes as much as 10 per cent. of glycogen. This observation is confirmed by KÜLZ ('Ztsch. f. Biol.,' vol. 22, p. 178). KÜLZ claims for his method in the case of the liver no greater accuracy than BRÜCKE's. He says (p. 193): "Sie gibt bei der Leber mindestens ebenso gute und beim Muskel entschieden bessere Resultate."

Seeing that the results given are no better than those obtained by simply boiling, the latter method was preferred, because it was found that the enormous proteid precipitate thrown down by the mercuric potassic iodide in KÜLZ's process required such prolonged and copious washing that the volume of the filtrate was inconveniently large, and that enormous quantities of alcohol were required for the subsequent precipitation of the glycogen.

To remove the glycogen as completely as possible, the proportion of water used was always large in relationship to the amount of liver substance. A piece of liver of from 5 to 10 grms., finely pounded after the initial boiling, was boiled in a vessel containing over a litre. The boiling was continued for many hours—usually, at least, twelve, the vessel being filled as the water evaporated. Three times in the course of the extraction the extract was passed through a linen filter and the pounded liver well squeezed. The residue, on being treated with caustic potash, and then precipitated with mercuric potassic iodide, gave only occasionally a faint trace of the glycogen reaction with iodine.

The voluminous filtrate was evaporated to about 200 cub. centims., treated with hydrochloric acid and mercuric potassic iodide, and filtered. The precipitate was well washed with water containing mercuric potassic iodide and acid. To the filtrate four times its volume of methylated spirits was added; and, after twenty-four hours, the precipitate was brought on a dried and weighed filter paper, and washed first with 60 per cent. alcohol, secondly with methylated spirit, thirdly with absolute

alcohol, then with ether, and finally with absolute alcohol. It was then dried at 110° C. The ash was not determined. FRAENKEL's ('Pflüger's Arch.,' vol. 52, p. 125) recently introduced method was tried, but was found to yield unsatisfactory results.

It may be urged that KÜLZ's observations point to so many fallacies in connection with any method of glycogen determination as to render the results of little value. But it must be remembered that, if the determinations are made under precisely the same conditions, the deficit will be fairly constant. The actual results obtained seem to indicate that the method is practically much more accurate than it appears on theoretical consideration. This is well shown by these experiments, in which both the glycogen and sugar were determined.

EXPERIMENT 18.

	Time after death.	
	2 minutes.	24 hours.
Glycogen	5·88	0·55
Glucose	trace of reduction	5·29
Total carbohydrates . . .	5·88	5·84

EXPERIMENT 19.

	Time after death.						
	2 minutes.	45 minutes.		135 minutes.		315 minutes.	
		Chloro-form.	No chloro-form.	Chloro-form.	No chloro-form.	Chloro-form.	No chloro-form.
Glycogen . . .	7·09	5·68	6·23	5·00	5·60	4·60	5·42
Glucose	0·23	1·39	0·98	1·96	1·66	2·53	1·88
Total carbohy-drates	7·09 to 7·32	7·07	7·21	6·96	7·20	7·18	7·20

An objection to every method of determining glycogen by precipitating it with alcohol is that some of the dextrins may also be in part thrown down, and be reckoned along with the glycogen. It would, perhaps, therefore, be better to call the results obtained " carbohydrates precipitated by 60 per cent. of alcohol," instead of " glycogen," but with this proviso it is needless to use such a cumbrous phrase.

When the sugar was also determined, the following method was employed. One half of the aqueous extract of the liver was heated and to it perchloride of iron in

sufficient amount to precipitate both proteids, and glycogen was added along with acetate of soda. The mixture was then neutralized with a solution of carbonate of soda and the clear watery fluid tested for glycogen by acidifying and adding iodine. If glycogen was present, the process was repeated. The clear fluid was then filtered off, the precipitate being well washed with hot water and the washings united to the filtrate.

This was then evaporated to a convenient volume, and the sugar determined volumetrically *as glucose*, by means of FEHLING's solution, sometimes with the addition of ferrocyanide of potassium (CAUSSE, 'Journal de Pharmacie et de Chimie,' 1889. DASTRE, 'Arch. de Physiol.,' 1891).

The following experiments were performed on the rate of conversion of hepatic glycogen.

<center>EXPERIMENT 1.   6.2.93.</center>

Rabbit killed at 1.5 P.M. Liver divided into three large pieces, A, B, C.
A weighed 5·1 grms. placed in boiling water at 1.6 P.M.
B „ 11·1   „   „   1.10 P.M.—4 minutes later.
C „   5·5 grms., placed in 0·75 per cent. NaCl solution, and kept at 35° to 39° C. till 5 P.M. (236 minutes), then boiled. Glycogen estimated by BRÜCKE's method.

$$
\begin{aligned}
\text{A.} \quad &\text{Glycogen} = 0\cdot195 \text{ grm.} = 3\cdot82 \text{ per cent.}\\
\text{B.} \quad &\text{\quad\quad\,\,} = 0\cdot333 \text{ ,,} = 3\cdot00 \text{ ,,}\\
\text{C.} \quad &\text{\quad\quad\,\,} = 0\cdot119 \text{ ,,} = 2\cdot16 \text{ ,,}
\end{aligned}
$$

In first 4 minutes 0·82 grm. of glycogen per 100 parts of liver disappeared, equal to 2·05 in 10 minutes.

In next 236 minutes 0·84 grm. of glycogen per 100 parts of liver disappeared, equal to 0·035 in 10 minutes.

<center>EXPERIMENT 2.   10.2.93.</center>

Rabbit killed at 1.15 P.M. Liver divided into several parts, A, B, C, D.
A weighed 8·4 grms., placed in boiling water at 1.17 P.M.
B „   8·6 grms., placed in 1·5 per cent. NaCl.
C „   10·1   „   „   „
D „   8·7   „   „   „
B kept at 40° C. till 2 P.M., then boiled.
C „   „   3.30 P.M., then boiled.
D „   „   6.30 P.M.,   „
Glycogen estimated by BRÜCKE's method, one half of the aqueous extract of each being taken for the purpose. The other half was treated by SCHMIDT MÜLHEIM's method, and the glucose estimated by means of FEHLING's solution without the addition of ferrocyanide of potassium. The end reactions were not well marked.

<center>*Glycogen.*</center>

$$
\begin{aligned}
\text{A.} \quad &\text{Glycogen} = 0\cdot292 \text{ grm.} = 7\cdot00 \text{ per cent.}\\
\text{B.} \quad &\text{\quad\quad\,\,} = 0\cdot268 \text{ ,,} = 6\cdot23 \text{ ,,}\\
\text{C.} \quad &\text{\quad\quad\,\,} = 0\cdot281 \text{ ,,} = 5\cdot6 \text{ ,,}\\
\text{D.} \quad &\text{\quad\quad\,\,} = 0\cdot233 \text{ ,,} = 5\cdot42 \text{ ,,}
\end{aligned}
$$

Glucose.

	Glucose in grms. in liver taken.	Per cent. of glucose in liver.
A.	less than 0·01	less than 0·23
B.	0·042	0·98
C.	0·083	1·66
D.	0·081	1·88

In first 45 minutes 0·86 grm. of glycogen per 100 parts of liver disappeared, equal to 0·19 in each 10 minutes.

In the next 90 minutes 0·63 grm. disappeared, equal to 0·07 per 10 minutes.

In the next 180 minutes 0·18 grm. disappeared, equal to 0·01 per 10 minutes.

EXPERIMENT 3.   13.2.93.

Rabbit killed at 1.23 P.M.   Liver cut up into A, B, and C.

A weighed 6·2 grms., placed in boiling water at 1.25 P.M.

B   „   4·0   „   „   „   1.35 P.M., 10 minutes later.

C   „   5·1   „   0·75 per cent. NaCl solution and kept at 37° C. till 3 P.M., then boiled.

Glycogen estimated by BRÜCKE's method.

A. Glycogen = 0·405 grm. = 6·53 per cent.

B.   „   = 0·239 „ = 5·97   „

C.   „   = 0·279 „ = 5·47   „

In first 10 minutes 0·58 grm. of glycogen per 100 parts of liver disappeared.

In the next 85 minutes 0·5 grm. disappeared, equal to 0·058 per 10 minutes.

EXPERIMENT 4.   13.1.93.

Rabbit killed at 12.58 P.M.   Liver minced and divided into A, B, C, D, and E.

A weighed 10·6 grms., placed in boiling water at 1 P.M.

B   „   10   „   0·75 per cent. NaCl solution at 1.1 P.M.

C   „   11·2   „   „   „   „   1.2 P.M.

D   „   11·9   „   „   „   „   1.3 P.M.

E   „   11·0   „   „   „   „   1.4 P.M.

B kept at 40° C. for 1 hour (60 minutes).

C   „   „   2 hours 35 minutes (155 minutes).

D   „   „   4 „ 15 „   (255   „   )

E   „   „   6 „ 15 „   (375   „   ).

Glycogen extracted by KÜLZ's method.

A. Glycogen = 1·164 grm. = 10·981 per cent.

B.   „   = 1·025 „ = 10·250   „

C.   „   = 1·095 „ = 9·776   „

D.   „   = 1·155 „ = 9·664   „

E.   „   = 1·004 „ = 9·127   „

Cultures on agar-agar from each gave free growth of micro-organisms, E had very strong smell.

In first 60 minutes 0·730 grm. of glycogen per 100 parts liver disappeared, or 0·122 per 10 minutes.

In next 95 minutes 0·273 grm. disappeared, or 0·03 per 10 minutes.

In next 100 minutes 0·112 grm., or 0·0112 per 10 minutes.

In next 120 minutes ·537 grm., or 0·045 per 10 minutes.

<div align="center">EXPERIMENT 5.   3.5.93.</div>

Rabbit killed at 12.35 P.M.   Liver divided in A, B, C.

A weighing 10 grms., was placed in boiling water at 12.29 P.M.

B   ,,   8   ,,   ,,   ,,   12.32 P.M.

C   ,,   9·3   ,,   ,,   ,,   12.42 P.M.

Glycogen by Brücke's method.

<div align="center">

A. Glycogen = 0·861 grm. = 8·61 per cent.

B.   ,,   = 0·669 ,,   = 8·36   ,,

C.   ,,   = 0·689 ,,   = 7·4   ,,

</div>

In first 3 minutes 0·25 grm. of glycogen per 100 parts of liver disappeared, or 0 83 grm. per 10 minutes.

In next 10 minutes 0·90 grm. of glycogen per 100 parts of liver disappeared, or 0·9 grm. per 10 minutes.

	Number of experiment.	Time in minutes from commencement.	Loss of glycogen per 100 parts liver.	Loss of glycogen per 100 parts liver per 10 minutes.
1st hour	5	3	0·25	0·85
,,	1	4	0·85	2·05
,,	3	10	0·58	0·58
,,	5	13	1·21	0·93
,,	2	45	0·86	0·19
,,	4	60	0·731	0·122
2nd hour	3	95	0·5	0·05
3rd ,,	4	155	1·49	0·07
4th ,,	1	240	1·66	0·069
5th ,,	4	255	1·205	0·051
6th ,,	2	315	1·67	0·05
,, ,,	4	375	1·855	0·049

In the next experiment the increase in glucose as indicated by the reducing power of the aqueous extract of the liver was estimated.

<div align="center">EXPERIMENT 6.   23.1.93.</div>

Rabbit killed at 12.55 P.M.   Liver divided into pieces A, B, C, and D.

A weighed 6·2 grms., placed in boiling water at 1 P.M.

B   ,,   6·1   ,,   ,,   0·75 per cent. NaCl solution, sterilized.

C   ,,   7·2   ,,   ,,   0·75   ,,   ,,   ,,

D   ,,   6·6   ,,   ,,   0·75   ,,   ,,   ,,

<div align="center"></div>

B kept at 40° C. till 2 P.M. (1 hour).
C   „   „   5 P.M. (4 hours).
D   „   „   10 P.M. (9   „   ).
Glucose extracted as in Experiment 2.

 Filtrate of A. Glucose = 0·593 per cent.
  „ B. „ = 0·175 grms. = 2·87 per cent. in liver.
  „ C. „ = 0·26 „ = 3·61 „
  „ D. = 400 cub. centims. (underwent fermentation before glucose was estimated).

Glucose formed per 10 minutes :—

    During first hour . . . . 0·362 per 100 parts of liver.
    From first to fourth hour . . . . . 0·04 „ „

Cultures on glycerine agar from B and C gave no growth of micro-organisms.

These experiments clearly show that the great and active disappearance of glycogen in the liver, kept in normal saline at the body temperature, is during the first half-hour, that the rate of conversion steadily diminishes during the first hour, and that after two hours it goes on at a very slow rate indeed.

The next point to be investigated is the relationship of the liver cells to these changes.

This may be studied in two ways :—

1st. By observing the influence of the destruction of the structure of the liver cells on the amylolysis.*

2nd. By investigating the changes which take place in the cells of the liver kept under the conditions above described.

B. INFLUENCE OF DESTRUCTION OF THE MORPHOLOGICAL STRUCTURE OF THE LIVER CELLS ON HEPATIC AMYLOLYSIS.

To destroy the cells, without in any way interfering with the possible action of any soluble ferments, the method of thoroughly rubbing a piece of liver up with fine clean sand was employed. The rabbit was killed in the usual manner and then pieces of the liver were taken. One of these was rubbed with sand till the whole became of a fine uniform cream-like consistence, in which microscopic examination revealed no structure. This was placed in about 150 cub. centims. of ·75 per cent. salt solution, at from 37° to 40° C. Another portion, roughly minced, was at the same time put into an exactly similar solution, while the third part was at the same moment thrown into boiling water. The first two portions were kept in the incubator for varying periods, and were then boiled and the glycogen extracted, as above described.

 * Throughout this paper amylolysis is used as a convenient abbreviation for "conversion of glycogen to glucose."

Rabbit killed at 1.22 P.M. Liver divided into parts A, B, C.

A weighed 5·7 grms., rubbed with sand in mortar and placed in 0·75 salt solution at 37° C., at 1.35 P.M.

B weighed 6·3 grms., roughly minced and placed in salt solution as above at 1.35 P.M.

C weighed 4·0 grms., placed in boiling water at 1.35 P.M.

A and B kept at 37° C. till 3·10 P.M. = Glycogen by BRÜCKE's method.

A. Glycogen = 0·312 grm. = 5·47 per cent. (slight loss due to cracking of beaker from bumping caused by sand).

B. Glycogen = 0·278 grm. = 4·41 per cent.

C. „ = 0·239 „ = 5·97 „

Rabbit killed at 11.42 A.M. Liver divided into parts A, B, and C.

A weighed 11·3 grms. pounded with sand as in last experiment, and placed in salt solution at 37° C., at 11.48 A.M.

B weighed 10·7 grms. minced and placed in salt solution, as above, at 11.48 A.M.

C weighed 8·6 grms. placed in boiling water at 11.48 A.M.

A and B kept at 37° to 40° C., till 3.50, and then boiled.

A. Glycogen = 0·572 grm. = 5·061 per cent.

B. „ = 0·25 „ = 2·336 „

C. „ = 0·453 „ = 5·267 „

Experiment.	Check.	Minced.	Pounded.	Time.
				hrs. min.
VII.	5·97	4·41	5·47 +	1 48
VIII.	5·267	2·336	5·061	4 8

The results of these two experiments are so fully confirmatory of one another that it was not considered necessary to extend the series. They show very clearly *the enormous diminution in the amylolysis induced by destroying the structural integrity of the liver cells. But at the same time they show that, though retarded, the conversion of glycogen is not completely stopped.*

C. STRUCTURAL CHANGES TAKING PLACE IN CELLS OF LIVER KEPT IN 0·75 SALT SOLUTION AT THE BODY TEMPERATURE.

The cellular death of an organ must be a slow and gradual process, not only as regards the individual cells in which the vital chemical changes probably slowly diminish and disappear, but also as regards the cells in mass, since experience shows that certain cells cease to act sooner than others.

It is impossible, by the microscopic examination of the cells, to say when the chemical changes, which constitute their life, have ceased. But it appeared probable that as these chemical changes diminished, the reaction of the cell to certain re-agents might alter, or that soon after the chemical changes had ceased, disintegrative changes might manifest themselves in the cell.

I. EHRLICH's researches ('Cbt. f. d. med. Wissen.', 1885, p. 113) induced me to try if, in methyl blue, a re-agent might be found which would indicate the cessation of active chemical changes.

For this purpose the reactions of this substance with ciliated epithelial cells and with leucocytes—the metabolic activity of which is so clearly indicated by movements—were studied. (α) Scrapings from the palate of a recently killed frog were spread on cover glasses and mounted in a ·4 per cent. solution of methyl blue in 0·75 per cent. salt solution.

It was found that the cells exhibiting active ciliary movement remained unstained, while those in which the ciliary movement had ceased, and the connective tissue cells, were all markedly stained—the nucleus being of a deep blue, the protoplasm of a paler blue. As the ciliary movement diminished and stopped, the previously active and unstained cells became blue. (β) The leucocytes of the frog's blood mounted in this solution remained uncoloured for fifteen or twenty minutes and then stained in the same manner as the ciliated cells.

In the case of the liver, when scrapings of the organ just excised from frogs or rabbits were treated with methyl blue in 0·75 per cent. salt solution in the cold, the cells and nuclei stained at once and deeply.

When the cells from the rabbit were treated with methyl blue solution at a temperature of 37° C. and examined in the warm stage at 37° C. the staining of the cells occurred just as in the cold.

The metabolic processes in liver cells, therefore, seem too slow to be indicated by their reaction with this colouring matter.

II. Another manner in which the cessation of active chemical changes might be made manifest is the occurrence of structural changes in the cells. Of course the onset of such changes may be the result of alterations in the metabolic processes or it may mark the commencement of post-mortem disintegrative changes.

The examination of liver cells at different periods after the excision of the organ, whether it be kept under the conditions described on p. 239 or simply allowed to lie in the room, shows the development of marked and interesting modifications in the structure of the cells.

Methods.

1. A fresh section of the liver was scraped and cover-glass preparations of the scraping made in the usual manner. These were examined :—

    α. Unstained in 0·75 per cent. normal saline solution.

β. Stained in 0·4 per cent. solution of methyl blue in 0·75 saline.

γ. Stained in LUGOL's iodine iodide of potassium solution.

2. Similar cover-glass preparations were fixed in absolute alcohol and then stained in hæmatoxylin and eosin, mounted in balsam.

3. Pieces of the liver were hardened in absolute alcohol, embedded in paraffin and sections prepared in the usual way, and stained with hæmatoxylin and eosin or with EHRLICH-BIONDI's solution.

The specimens were studied with a LEITZ 7 objective, a ZEISS F., and a ZEISS apochromatic oil immersion, 3 m.m.

The examination of fresh cover-glass preparations stained with methyl blue is of special value in demonstrating the changes in the cell-substances, while the nuclear changes are more clearly seen in specimens hardened in alcohol and stained with hæmatoxylin and eosin.

The structure of the liver cell has been described by various histologists. LANGLEY ('Roy. Soc. Proc.,' vol. 34, p. 20) gives some account of the previous work upon the subject, and, as the result of his own investigations, describes the cell protoplasm as consisting of a network, the meshes of which, throughout the cell, being of much the same size, and the outer part of the cell being formed by a thin layer of slightly modified protoplasm. The interfibrillar paraplasm he describes as consisting of—

1. Spherical granules, probably proteid in nature; 2. Spherical globules of fat; 3. Hyaline substance filling up spaces not occupied by granules. He does not state whether this description applies to the living cell or to cells after hardening by the methods described in the paper.

The description and figures of the structure of the liver cell given in vol. 4 of the 5th edition of FOSTER's 'Physiology' are founded upon these observations of LANGLEY.

DELÉPINE ('Roy. Soc. Proc.,' vol. 49, p. 64), describes LANGLEY's "protoplasm network" as the mytoma, and calls the fluid interstitial substance the paramytoma. The term paraplasm he reserves for the results of the cellular metabolism, whether these be dissolved in the paramytoma or suspended in it as granules, globules, crystals, &c.

Setting aside all theories, what can be observed in the fresh living liver cell stained with methyl blue is a fine close network of fibres, which takes up the re-agent, and an interstitial material which does not stain. The network in the living cell is so close and fine that the protoplasm appears to be almost uniformly stained of a pale grey-blue. In the interstitial material, fat globules may sometimes be seen, and in specimens from well-nourished animals, stained with iodine, this material, either throughout the whole cell, or round the nucleus, or towards one or other margin, becomes of a mahogany-brown colour.

In fresh living cells I have been unable to see the granules described by LANGLEY. These are well seen in hardened specimens.

Most commonly there are two nuclei in each cell. These are large and circular, with a well-defined nuclear membrane, and a marked chromatin network, and one or more nucleoli. I have never, in a healthy adult liver cell, seen a mytotic nucleus.

The rapidity with which changes occur in the cells of the excised liver, when simply kept in the room or placed in a moist atmosphere in the incubator at 40° C., or when kept in 0·75 per cent. saline solution at 40° C., varies enormously in different animals.

In the cells of the liver kept at the ordinary room temperature, 16° C., no marked changes occur for 12 or 24 hours. If the organ is placed in the incubator, changes become manifest much earlier—within three or four hours. When the tissue has been in salt solution in the incubator changes may sometimes be made out within an hour, though frequently they are delayed for two or more hours.

The changes which occur are the same in all cases.

The first alteration is in the intra-cellular network. This becomes more manifest, giving the cells a more granular appearance. After two hours, in specimens kept in salt solution, this change is usually very marked. At first it was thought that this was due to the imbibition of fluid from the surrounding solution. But the same changes occur in the cells of the liver when not placed in solution, but kept dry.

The change once commenced advances slowly. The network becomes coarser, and becomes broken up. It still stains intensely with methyl blue, and the cell now appears as a clear, colourless structure with masses of the blue-stained network in it. These tend to collect round the nucleus. The nuclear changes are usually later in occurring, though in one or two cases I have observed them fairly early. The nucleus loses its sharp outline, loses its distinct network, and stains more diffusely blue with hæmatoxylin. After some hours it begins to break down and to appear as masses of material throughout the cell, stained partly blue with hæmatoxylin, partly red with eosin.

Speaking generally, it may be said that these changes manifest themselves, in the liver cells kept at 40° C. in saline solution, sometimes within the first hour, and that they become very marked within three hours.

In specimens placed in salt solution rendered faintly alkaline with carbonate of soda the rapidity of the early changes is not as a rule markedly delayed, but the late changes may be postponed for a very considerable period.

In a subsequent part of this paper it will be shown that the presence of chloroform greatly accelerates the initial conversion of glycogen to glucose, but that it has no action on the slow secondary conversion. In this connection it is important to notice that the presence of chloroform enormously accelerates the changes just described as occurring in the liver cells. In a liver kept in saline solution at 40° C., through which a stream of chloroform vapour is passed, the changes in the liver cells are at the end of half an hour as far advanced as they are in a non-chloroform specimen

after three or four hours. In chloroform livers, at the end of three hours, the intra-cellular network and the nucleus are both in an advanced state of disintegration.

Chloroform markedly hastens these changes in the liver cells.

These observations, taken in conjunction with the last, show that *the amylolysis may be divided into an early more rapid stage before and accompanying disintegra-tion of the liver cells, and a late slower stage after disintegration of the cells.*

The difference between these two stages in the amylolysis is further supported by the influence of various other agents.

D. Influence of Various Factors on Hepatic Amylolysis.

1. *Temperature.*

At one time it was pretty universally held that by the influence of temperature the living action of cells might be distinguished from the chemical action of soluble ferments.

Hofmeister, in his researches on the changes of peptone in the intestinal mucous membrane, found that a temperature of 60° C. arrested these changes, and concluded that this indicated that they were dependent on the life of the cells, which he supposed was destroyed by such a temperature, and not to a zymin which should have acted even after exposure to a higher temperature.

While undoubtedly certain cells of lower organisms do resist for some time a temperature as high as 60° C., I believe Hofmeister is right in concluding that the life of the cells of the mammalian body is destroyed by such a temperature. But it must be remembered that many ferments, under certain conditions, have their action, in part or in whole, abolished by exposure to even a lower temperature than 60° C.

For these reasons, the influence of temperature on hepatic amylolysis is by no means conclusive as to its nature.

The following two experiments seem to show that the extensive early amylolysis is stopped by exposure of the liver to a temperature of 60° C. for an hour, while the later slower change is allowed to progress :—

Experiment 9. 22.5.91.

Rabbit killed at 2.24. Liver cut into A, B, and C.

A weighing 12·8 grms., was placed at once in boiling water.

B   „   13·5   „   „   in 0·75 per cent. NaCl at 60° C.

C   „   11·7   „   „   „   „   „   40° C.

B and C were kept at these temperatures till 3.25, when B was cooled to 40° C. and left at this temperature with C till 6.15, when both were boiled.

Glycogen by Brücke's method.

A. Glycogen = 0·103 grm. = 0·804 per cent.

B.   „   = 0·093. „   = 0·69   „

C.   „   = 0·023 „ = 0·196   „

Rabbit killed at 11.37.   Liver cut into A, B, and C.

A weighing 12·7 grms., placed at once in boiling water.

B   „   11·3   „   „   in 0·75 per cent. NaCl at 60° C.

C   „   11·1   „   „   „   .   „   „   40° C.

B and C kept at these temperatures till 12.20 then cooled to 40° C. and kept with C at this till 4.30, when both were boiled.

Glycogen by BRÜCKE's method.

A. Glycogen = 0·104 grm. = 0·811 per cent.

B.   „   = 0·079 „   = 0·700   „

C.   „   = 0·050 „   = 0·450   „

The cells of the liver, subjected to a temperature of 60° C. for about an hour, show a peculiar change very different from that so rapidly produced by chloroform, and which occurs more slowly in livers kept at 40° C. Here the protoplasm becomes filled with minute granules, apparently due to precipitation of proteids.

2. *Fluoride of Sodium.*

Within the last year ARTHUS and HUBER ('Arch. de Physiol.,' 5th series, vol. 4, p. 651) have maintained that a 1 per cent. solution of fluoride of sodium destroys the activity of living protoplasm, but does not interfere with the actions of enzymes. They give experiments on the influence of this substance on the post mortem production of sugar in the liver as estimated by the amount of sugar present after periods of twenty-four hours and more, which show that the production of sugar is not stopped. They say " On peut donc conclure que la transformation du glycogène en sucre dans le foie séparé de l'organisme est un phénomène de fermentation par ferment soluble."

With this substance I have made two experiments which clearly show that the early rapid amylolysis is retarded, although the later slower conversion may be allowed to go on as shown by ARTHUS and HUBER.

Rabbit killed at 11.38.   Liver divided into A, B, and C.

A weighed 3·0 grms., placed in boiling water at 11.40.

B   „   5·1   „   „   0·75 per cent. NaCl at 40° C. at 11.41.

C   „   5·6   „   „   1·0   „   fluoride of sodium solution at 40° C. at 11.42.

B and C kept at 40° C. till 1.38, then boiled.

Glycogen by BRÜCKE's method.

A. Glycogen weighed 0·246 grm. = 8·22 per cent.

B.   „   „   0·270   „   = 5·29   „

C.   „   „   0·459   „   = 8·19   „

This experiment is not quite satisfactory since fluoride of sodium is not fully soluble in 60 per cent. of alcohol.

For this reason the influence of the fluoride on the amount of sugar produced was studied. -

EXPERIMENT 12.   7.6.93.

Rabbit killed at 10.45 A.M.   Liver divided into A, B, and C.

A weighed 15 grms. in boiling water at 10.47.

B   „   10·48 grms. in 1 per cent. NaCl at 10.48.

C   ,,   10·49   „   „   „   fluoride of sodium at 10.49.

B and C kept at 37° to 40° C. till 11.50, then boiled.   Glucose as in previous experiments.

	Glucose in extract.	Glucose per cent. in liver.
A.	0·068	0·43
B.	0·196	1·63
C.	0 138	0·86

This substance does not accelerate the disintegration changes in the liver cells described on p. 248.

3. Chloroform.

Early in the investigation, the use of chloroform water, to distinguish between the vital action of cells and the influence of a soluble ferment, was suggested by a first glance at SALKOWSKI's paper "Ueber Autodigestion der Organe" ('Ztsch. f. klin. Med., Sup. Jubelheft,' 1890, p. 77). In this he maintains that, while chloroform water entirely prevents the action of living cells by stopping their metabolism, it in no way interferes with the action of the soluble zymins.[*]   He gives one experiment to show that the amylolytic ferment continues to act in chloroform.

EXPERIMENT 3.   (p. 90.)

Liver divided into two parts of 23 grms.

A. in 400 cub. centims. of chloroform water.

B. sterilized by boiling, then put in 440 cub. centims. of chloroform water.   Both were digested for 68 hours.

In solution.	A.	B.
Sugar	Abundant.	Trace.
Glycogen.	None.	Abundant.

The quantitative determination of sugar gave in 1000 grm. liver,

A. 48·23

B.   3·65

[*] MÜNTZ ('Compt. Rend.,' 1875, p. 1251) had fifteen years previously stated that chloroform has this action.

He concludes this experiment by saying, "Bezüglich des Glycogens bestätigt also der Versuch die geläufige Anschauung, dass die Umwandlung desselben in Zucker von einem Enzym abhängt, im Gegensatz zu Dastre, welcher kürzlich zu dem Resultat gelangt ist, dass dieser Process von dem Protoplasma der Leberzellen abhängt."

Such an experiment in no way justifies his conclusions.

Further study of this method (FOKKER, 'Fortsch. d. Med.' 1890, No. 3), as admitted by SALKOWSKI (*ibid.*, 1890, No. 5), shows that chloroform greatly diminishes the activity of many unorganized ferments, and therefore cannot be considered of much value in the differentiation of zymin action from the vital action of protoplasm.

The curious and unexpected result obtained in a preliminary experiment, and the statement that chloroform administration is followed by the occurrence of glycosuria (HILTON FAGGE, 'Principles and Practice of Medicine,' vol. 2, p. 414), as well as the experience of DR. STOCKMAN, who tells me that he has frequently, after the administration of chloroform to rabbits and dogs, found a fermentable and reducing substance in the urine, induced me to study more fully the action of chloroform on hepatic amylolysis.

That glycæmia as well as glycosuria is induced by chloroform has been shown by OTTO ('Pflüger's Arch.,' vol. 35, p. 467). He estimated before and after the adminis-tration of morphin, chloral, and chloroform, the reducing substances in the blood before and after fermentation, and found that morphia causes a slight increase in the glucose, but a marked increase in the not fermentable reducing substances; that chloral has little action on the glucose, but markedly increases the other reducing substances, while chloroform has the same action as morphin, but more marked.

Method.

To test the action of this substance upon the changes in the liver, the animal was killed as before described, the liver excised, minced and divided into three portions, which were weighed; one was instantly plunged into boiling water. The other two were placed in bottles containing 0·75 per cent. salt solution at from 37° to 40° C. Through one bottle a stream of air and through the other a stream of chloroform vapour were conducted by means of a pump, both bottles being kept in the incubator. At the end of varying periods the contents of both bottles were boiled and the glycogen extracted.

<div align="center">EXPERIMENT 13.   28.5.91.</div>

Rabbit killed at 2.28 P.M.   Liver minced and divided into three parts, A, B, and C.

A weighed 6·1 grms., placed in boiling water at 2.34.

B   „   6·2   „   „   150 cub. centim., 0·75 per cent. NaCl solution with stream of chloroform vapour at 2.35.

C weighed 5·9 grms., placed in 150 cub. centims., 0·75 per cent. NaCl solution with stream of air at 2.35.

B and C kept at 40° C. till 6.15 P.M. (3 hrs. 40 min.).

Glycogen extracted by BRÜCKE's method.

A. Glycogen = 0·201 grm. = 3·295 per cent.
B.   „   = 0·060 „   = 0·096   „
C. No precipitate with alcohol.
        Glycogen = 0·00 per cent.

EXPERIMENT 14. 3.6.91.

Rabbit killed at 11.37 A.M. Liver minced and divided into three parts.
A weighed 12·7 grms., placed at once in boiling water.
B   „   10·3 „   „ in 150 cub. centim. of 0·75 per cent. NaCl solution with stream of chloroform.
C weighed 11·1 grms., placed in 150 cub. centim. of 0·75 per cent. NaCl solution with stream of air.
B and C kept at 40° C. till 4.30 P.M. (5 hours).
Glycogen extracted by BRÜCKE's method.

A. Glycogen = 0·104 grm. = 0·811 per cent.
B.   „   = 0·017 „   = 0·165   „
C.   „   = 0·50   „   = 0·450   „

EXPERIMENT 15. 9.11.91.

Rabbit killed at 12.30 P.M. Liver divided into two parts A and B.
A weighed 25·2 grms., placed in 0·75 per cent. NaCl. solution with stream of air.
B   „   23·1 „   „   „   „   „   „   „   „   chloroform vapour.
Kept at 40° C. till 3.30 (3 hours).
Glycogen extracted by BRÜCKE's method.

A. Glycogen = 1·012 per cent.
B.   „   = 0·735   „

EXPERIMENT 16. 22.8.92.

Rabbit killed at 11.30 A.M. Liver minced and divided into three parts.
A weighed 17·6 grms., placed in boiling water.
B   „   15·0 „   „   0·75 per cent. NaCl solution with stream of air.
C   „   15·8 „   „   „   „   „   „   „   „   chloroform vapour.
B and C kept at 32° to 42° C. till 3.30 (4 hours).
Glycogen extracted by BRÜCKE's method.

A. Glycogen = 0·37 grm. = 2·102 per cent.
B.   „   = 0·291 „   = 1·94   „
C.   „   = 0·134 „   = 0·848   „

That such results are not peculiar to the rabbit, or to herbivorous animals, is shown by the following experiment on the cat :—

A large fat cat was killed by chloroform and bleeding. The liver was excised, minced, and divided into portions A, B, and C.

A weighed 9·5 grms. placed in boiling water at 3.35 P.M.

B   ,,   10   ,,   in 0·75 per cent. NaCl with chloroform.

C   ,,   11·4 ,,   ,,   ,,   ,,   without ,,

B and C kept at 37° to 41° C. till 9.10 P.M., then boiled.

         A. Glycogen = 0·197 grm. = 2·07 per cent.

         B.   ,,   = 0·073   ,,   = 0·73   ,,

         C.   ,,   = 0·111   ,,   = 0·97   ,,

SUMMARY.

Experiment.	Check.	Chloroform.	No chloroform.	Remarks.
13	3·295	0·000	0·096	40° C. for 4 hours
14	0·811	0·165	0·450	,, 5 ,,
15	Lost	0·735	1·012	,, 3 ,,
16	2·102	0·848	1·94	,, 4 ,,
17	2·07	0·73	0·97	

This series of experiments clearly shows that *in the excised liver the amylolysis is enormously increased by the presence of chloroform.*

That the glycogen is converted almost entirely into glucose is shown by the following experiment.

Rabbit killed at 12.20. Liver minced and divided into two, A and B.

A weighed 12·5 grms.; placed in boiling water at 12.28.

B   ,,   22   ,,   placed in 0·75 per cent. solution of NaCl, with stream of chloroform vapour at intervals.

B kept at 40° C. till 12.30 on 12th. (24 hours).

Glycogen extracted by boiling as in previous experiments. Extract concentrated. Proteids and glycogen precipitated with excess of alcohol, and precipitate well washed with 70 per cent. alcohol. Filtrate evaporated to drive off alcohol, and glucose estimated by FEHLING's method. Filter paper and precipitate extracted for glycogen by BRÜCKE's method.

Glucose.

A. Filtrate = 300 cub. centims., with 1 centim. of FEHLING, only a trace of reduction.

B. Filtrate (α), First determination, = 1·22 grm. glucose.

         Glucose = 5·54 per cent. of liver.

     (β) Second determination, = 1·11 grm. glucose.

         Glucose = 5·045 per cent. of liver.

         Mean   = 5·292   ,,   ,,

Glycogen.

A. Glycogen = 0·735 grm. = 5·8 per cent.
B.   „   = 0·121 „ = 0·55 „

	Glycogen.	Glucose.	Total carbo-hydrates.
Before chloroform digestion . .	5·8	0	5·8
After „ „ . .	0·55	5·292	5·842

It now became necessary to investigate whether it is the initial rapid, or the subsequent slower amylolysis, or both, which are accelerated by the presence of chloroform.

For this purpose the following experiment was undertaken :—

EXPERIMENT 19.   10.2.93.

Rabbit killed at 1.15 P.M., and liver minced and divided into seven portions, A, B, b, C, c, D, d.
A weighed 8·4 grm. placed in boiling water at 1.17 P.M.
B   „   10·2 „   „   1·5 per cent. NaCl, with Chloroform at 37° C.
b   „   8·6 „   „   „   „   without „   „
C   „   9·9 „   „   „   „   with   „   „
c   „   10·1 „   „   „   „   without „   „
D   „   8·7 „   „   „   „   with   „   „
d   „   8·7 „   „   „   „   without „   „
B and b kept at 37° C. till 2   P.M., then boiled.
C and c   „   „   „ 3.30 P.M.   „
D and d   „   „   „ 6.30 P.M.   „
Aqueous extract divided into equal parts; in one glycogen determined by BRÜCKE's method, and in the other glucose determined by SCHMIDT MÜLHEIM's and FEHLING's methods.

Glycogen.

A. Glycogen = 0·292 grm. = 7·09 per cent.
B.   = 0·290   = 5·68
b.   = 0·268   = 6·28
C.   = 0·245   = 5·00
c.   = 0·281   = 5·60
D.   = 0·198   = 4·60
d.   = 0·233   = 5·42

	Glucose contained.	Glucose per cent. Liver.
A	— 0·01	— 0·23
B	0·071	1·39
b	0·042	0·98
C	0·097	1·96
c	0·083	1·66
D	0·111	2·68
d	0·081	1·88

	Glucose.		Glycogen.		Time.
	Glucose, per cent.	Gain per ten minutes.	Glycogen, per cent.	Loss per ten minutes.	
A	— 0·23	—	7·09	—	
B	1·39	0·308	5·68	0·31	} 45 minutes
b	0·98	0·218	6·23	0·19	
C	1·96	0·06	5·00	0·07	} 45 + 90 minutes
c	1·66	0·07	5·60	0·07	
D	2·58	0·036	4·60	0·02	} 45+90+180 minutes
d	1·88	0·012	5·42	0·01	

This experiment shows that it is *the early amylolysis which is accelerated under the influence of chloroform.*

That *the amylolysis, subsequent to the destruction of the cells, is not materially influenced by chloroform* is shown by the next two experiments.

<center>EXPERIMENT 20.   7.12.92.</center>

Rabbit killed at 12.55 P.M.   30 grms. of liver pounded in mortar with washed sand, extracted with 0·75 per cent. NaCl solution, and squeezed through calico.   1 grm. glycogen dissolved in 0·75 per cent. NaCl solution was added to the extract, and the whole made up to 210 cub. centims.   This was divided into:—

A.   70 cub. centims. placed at once in boiling water.

B.   70 cub. centims. placed in 0·75 per cent. NaCl with stream of chloroform vapour.

C.   70 cub. centims. placed in 0·75 per cent. NaCl with stream of air.

B and C kept at 40° till 9 P.M. (8 hours).

Glycogen extracted by BRÜCKE's method.

<center>

A.   Glycogen = 0·656 grm.

B.   „   = 0·465 · „

C.   „   = 0·499 „

</center>

Rabbit killed at 1 P.M. 32·7 grms. of liver pounded in mortar extracted with 0·75 per cent. NaCl solution, and filtered through cotton cloth. 1 grm. of glycogen dissolved in 5·70 per cent. NaCl solution was added to this extract, and the whole made up to 250 cub. centims. Of this,

A. 80 cub. centims. were placed at once in boiling water.

B. 80 cub. centims. treated with stream of chloroform vapour.

C. 80 cub. centims. treated with stream of air.

B and C kept at 40° C. till 5 P.M. (4 hours).

Glycogen extracted by BRÜCKE's method.

$$
\begin{aligned}
\text{A.} \quad & \text{Glycogen} = 0\cdot507 \text{ grm.} \\
\text{B.} \quad & \quad\quad\quad\; ,, \quad = 0\cdot480 \quad ,, \\
\text{C.} \quad & \quad\quad\quad\; ,, \quad = 0\cdot421 \quad ,,
\end{aligned}
$$

ACTION of Chloroform on Glycogen of Liver in Living Animal.

The practical importance of the glycæmia and glycosuria of chloroform poisoning induced me to undertake some observations on the effect of the prolonged administration of the drug on the glycogen of the liver of living animals.

To arrive at definite results from such experiments is by no means easy. In the first place, the glycæmia may be due to an increased conversion of glycogen, but this may be masked by a concomitant increased formation of the substance in the liver. Again, the impossibility of securing anything like an equality in the amount of glycogen in the liver of two or more animals, even when kept for long on the same diet and in the same conditions, diminishes the value of comparison between the liver of poisoned and unpoisoned animals.

ROHMANN ('Pflüger's Arch.,' vol. 39, p. 21), for his experiments on the influence of ammonia on the hepatic glycogen, used two rabbits kept on the same diet and under the same conditions for some time, to one of which ammonia was given while the other was used as a check; and these experiments indicate that with care the amount of glycogen in the livers of two animals may be made approximately the same.

My observations show that the glycogen in the liver of adult animals kept on the same diet and in the same conditions does not vary greatly in amount; provided always the animals are either maintaining their weight or gaining weight in the same proportion, and *that the period between death and boiling the liver is sufficiently short.*

This is indicated by the following experiments :—

Two young brown doe rabbits, procured on 26.4.87, and placed in separate cages, on diet of oats and water.

	Weight in grms.	
	A.	B.
26.4	1063	1134
29.4	1105	1134
30.4	1105	1134
2.5	1105	1077
3.5	1105	1105
5.5	1134	1134
6.5	1134	1105

The rectal temperature of both, on the 6th, was 39° C.

Both killed in the usual manner.

A. Liver, without gall bladder, weighed 39·3 grms., of which 30 grms. were taken for analysis.

B. Liver   ,,   ,,   ,,   weighed 29·72 grms., of which all was taken for analysis.

Several more minutes elapsed between the death of the animal and the boiling of the liver in B than in A.

Glycogen estimated by LANDWEHR's method ('Ztsch. f. physiol. Chemie,' vol. 8, p. 170).

Glucose estimated by evaporating the filtrate from the iron precipitate, and titrating with FEHLING's solution.

$$\begin{aligned}
\text{A.}\quad \text{Glycogen} &= 0{\cdot}24 \quad \text{grm.} = 0{\cdot}8 \quad \text{per cent.}\\
\text{Glucose} &= 0{\cdot}0526 \quad ,, \quad = 0{\cdot}175 \quad ,,\\
\text{Total carbohydrates} &= 0{\cdot}2926 \quad ,, \quad = 0{\cdot}975 \quad ,,
\end{aligned}$$

$$\begin{aligned}
\text{B.}\quad \text{Glycogen} &= 0{\cdot}15 \quad ,, \quad = 0{\cdot}5 \quad ,,\\
\text{Glucose} &= 0{\cdot}14 \quad ,, \quad = 0{\cdot}471 \quad ,,\\
\text{Total carbohydrates} &= 0{\cdot}29 \quad ,, \quad = 0{\cdot}971 \quad ,,
\end{aligned}$$

<div align="center">EXPERIMENT 23.</div>

Two young white doe rabbits, put on diet of oats and water, on May 25th, 1887.

. Date.	Weight, in grms.		Remarks.
	A.	B.	
27.5	708·7	623·7	Each took same amount
28.5	680·4	595·3	of food.

On May 28th they were killed in the usual manner, the blood being caught in a known volume of water.

A yielded 18 cub. centims. of blood.

Liver weighed 20·5 grms.

B yielded 18 cub. centims. of blood.

Liver weighed 18·7 grms.

Sugar of blood estimated by SEEGEN's method.

Glycogen of liver estimated by LANDWEHR's method, and sugar estimated as in last experiment.

A. *Blood*   Glucose = 0·106 per cent.
   Liver   Glycogen = 0·53   ,,
               Glucose = 0·231   ,,
     Total carbohydrates = 0·761   ,,

B. *Blood*   Glucose = 0·105   ,,
   Liver   Glycogen = 0·48   ,,
               Glucose = 0·300   ,,
     Total carbohydrates = 0·780   ,,

The results of the succeeding experiments fully confirm these observations.

If, however, the animals are not kept on a similar diet, and if they are not either steadily and uniformly gaining, losing, or maintaining weight, or if the greatest precautions are not taken to make the times between killing the animal and boiling the liver of short and equal duration, the greatest variations in the percentage of hepatic glycogen will be found.

In the following experiment the fullest precautions were taken to observe the above conditions :—

EXPERIMENT 24.

From a litter of nine young rabbits, three which were gaining weight were selected.

WEIGHT in Grms.

	5.9.92.	6.9.92.	7.9.92.
A	113	112	116
B	150	150	155
C	122	120	123

A was used as a check.

B was kept under chloroform from 11.30 to 2.20 when it was allowed to recover.

C had 0·01 grm. of bimeconate of morphin at 11.30 and 0·02 grm. at 12, but was never markedly under the influence of the drug.

A was killed in the usual way at 2.56 and the liver excised and thrown into boiling water at 2.58. It weighed 3·2 grms.

B was killed at 2.31 and the liver thrown into boiling water at 2.33. It weighed 5·2 grms.

C was killed at 3.25 and the liver thrown into boiling water at 3.27. It weighed 3·4 grms.

Glycogen extracted by BRÜCKE's method.

A. Weight of glycogen = 0·046 grm. = 1·437 per cent.
B.   = 0·039   = 0·75
C.   = 0·047   = 1·385

<div align="center">Experiment 25.</div>

From a litter of nine young rabbits, three which were losing weight were selected.

<div align="center">Weight in Grms.</div>

	·5.9.92.	6.9.92.	7.9.92.
A.	120	117	114
B.	100	98	96
C.	157	151	150

A was used as check.

B was kept under chloroform from 11.30 to 2.20 when it was allowed to recover.

C had 0·01 grm. of bimeconate of morphin at 11.20 and at 12. At 2 p.m. it was in a deep sleep.

A was killed at 3 and the liver was thrown into boiling water at 3.1. It weighed 3·2 grms.

B was killed at 3.34 and the liver thrown into boiling water at 3.36. It weighed 2·8 grms.

C was killed at 3.28 and the liver thrown into boiling water at 3.30. It weighed 4 grms.

Glycogen estimated by Brücke's method.

<div align="center">

A. Weight of glycogen = 0·012 grm. = 0·375 per cent.

B.   „   „   = 0·003 „   = 0·107   „

C.   „   „   = 0·016 „   = 0·40   „

</div>

<div align="center">Experiment 26.</div>

Two young rabbits were bought on October 16th, 1892, and were kept in same cage and on same food till November 9th, being weighed each day.

<div align="center">Weight in Grms.</div>

	17.10.	19.10.	22.10.	25.10.	28.10.	31.10.	3.11.	6.11.	9.11.
A . . .	1220	1300	1300	1413	1430	1490	1600	1550	1560
B . . .	970	1002	1070	1085	1097	1120	1230	1185	1200

No food was given after morning of 8th.

At 11.30 on the 9th, A was chloroformed and was kept under the drug till, at 3.30, death ensued. The abdomen was rapidly opened, and the hepatic vein cut to deplete the liver. The liver weighed 64 grm., of which 26·2 grm. were taken for analysis by Brücke's method.

<div align="center">Weight of glycogen = 0·436 grm. = 1·664 per cent.</div>

B was killed at 3.50. The liver weighed 54 grm., of which 25·7 grm. were taken for analysis by Brücke's method.

<div align="center">Weight of glycogen = 1·005 grm. = 3·91 per cent.</div>

URINE taken from Bladder *post mortem.*

	Yeast.	FEHLING.
A . . .	Marked fermentation	Marked reduction
B . . .	No ,,	Slight ,,

EXPERIMENT 27.

From young rabbits of one brood, bought on October 16th, 1892, and kept upon the same diet, being weighed daily till November 9th.

WEIGHT in Grms.

	17.10.	19.10.	22.10.	25.10.	28.10.	31.10.	3.11.	6.11.	9.11.
A . . .	1410	1424	1330	1532	1497	1550	1712	1660	1550
B . . .	1243	1245	1350	1325	1360	1440	1480	1460	1450
C . . .	1410	1450	1425	1432	1492	1520	1648	1625	1630
D . . .	1261	1240	1297	1262	1285	1352	1405	1360	1360

No food was given after the morning of the 8th November.

On November 9th, at 11.30, B, C, and D were chloroformed; B died suddenly at 12.30, C. died at 12.45. Immediately after death in each case the hepatic vein was opened, and the liver bled. D was killed at 3.40, and A at 4 P.M.

Liver of A = 51·5 grm., of which 20 were taken for analysis.

,,   B = 50   ,,   ,,   all was   ,,   ,,

,,   C = 54·5   ,,   ,,   27·5 were taken for analysis.

,,   D = 40·2   ,,   ,,   18·5   ,,   ,,

Analysis of glycogen by BRÜCKE's method.

A. Glycogen = 0·133 grm. = 0·665 per cent.

B.   ,,   = 0·358   ,,   = 0·716 - ,,

C.   ,,   = 0·16   ,,   = 0·582   ,,

D.   ,,   = 0·017   ,,   = 0·091   ,,

URINE taken from Bladder *post mortem.*

	Yeast.	FEHLING.
A . . .	No fermentation	Slight reduction
B . . .	,, ,,	,, ,,
C . . .	Slight fermentation	,, ,,
D . . .	Marked ,,	,, ,,

Four young pups of one litter were weighed on October 4th.

A = 443 grm.

B = 457 grm.

C = 501 grm.

D = 440 grm.

C was rejected as not corresponding to the others in weight.

D was put under chloroform at 11 P.M., and kept under till 3.6 P.M.   The liver weighed 18·4 grm., and all was taken for analysis.

A was killed at 3.12 P.M.   The liver weighed 18·1 grm., and was all analyzed.

B was killed at 3.21 P.M.   The liver weighed 18·3 grm., and was all taken for analysis.

Glycogen extracted by BRÜCKE's method.

A. Weight of glycogen = 0·258 grm. = 1·425 per cent.

B.   „   „   = 0·183 „   = 1·000   „

D.   „   „   0·203   = 1·103

URINE taken from Bladder *post mortem*.

	Yeast.	FEHLING.
A . . . B . . . D . . .	No fermentation „ „ Slight fermentation	No reduction „ „ Not enough to test

SUMMARY of Chloroform Experiments on Living Animals.

Experiment.	Kind of animal.	Check animal.	Chloroformed for 3 or 4 hours.
24	Rabbit	1·437	0·75
25	„	0·375	0·016
26	„	3·91	1·664
27	„	0·665	0·091
28	Dog	{ 1·425 { 1·000	1·103

These experiments, although they must be accepted with caution, seem to indicate that, *in the living animal under the influence of chloroform, glycæmia and glycosuria are produced by an increased conversion of glycogen to glucose.*

In Experiment 28, in which young dogs were employed, the result was negative.

NEBELTHAU ('Ztsch. f. Biol.,' vol. 28, p. 138) attempts to prove that chloroform, as well as chloral hydrate, chloralamid, paraldehyd, ether, alcohol, and sulphonal, increases the amount of hepatic glycogen, but his experiments disprove rather than prove his

contention. He employed hens starved for six days. KÜLZ had previously shown that, in these conditions, the amount of hepatic glycogen does not exceed 0·95 per cent. While, after the administration of chloral the hepatic glycogen was enormously increased from 1·22 per cent. to 5·12 per cent., the following results with chloroform show a *diminution* rather than an increase.

Duration of fast.	Amount of chloroform.	Duration of life after the first administration of chloroform.	Per cent. of glycogen in liver.
days.	cub. centims.	Hours.	
6	1·5	24	Trace.
6	0·5	24	0·42
6	1·5	26	0·60
6	1·5	18	1·49
6	1·25	14	1·40
6	1·0	15	0·68

Average = 0·76.

To what is this increased conversion due? Chloroform may act directly upon the liver cells, or, by impairing the oxidative changes in the liver.

That diminished oxidation does cause glycæmia and glycosuria has been shown by DASTRE and more recently by ARAKI ('Ztsch. f. physiol. Chem.,' vol. 15, 335 and 546) and by ZILLESEN (*ibid.*, p. 387).

According to LÉPINE and BARRAL ('Compt. Rend.,' 23 Juin, 1890) this is due to a diminution in the glycolytic power of the blood. They, however, made no experiments to exclude the possibility of there being, at the same time, an increase in the amylolysis in the liver.

That an imperfect supply of oxygen has no direct effect on the rate of hepatic amylolysis seems to be indicated by the following two experiments on the excised liver.

<div align="center">EXPERIMENT 29.</div>

Rabbit killed at 11 A.M. on 28.4.92. Liver cut up into A, B and C.

    A. Weighed 17·5 grms., placed in boiling water at once.

    B.   ,,   24·8 ,,   ,,   0·75 per cent. salt solution at 38° C.

    C.   ,,   24·5 ,,   ,,   ,,   ,,   ,,   ,,

A continuous stream of air was kept up through B, while C was left at rest. Both were kept at from 36° to 40° C. till 1 P.M. (2 hours), and then boiled.

Glycogen by BRÜCKE's method.

    A. Weight of glycogen = 1·639 grm. = 9·36 per cent.

    B.   ,,   ,,   = 1·175 ,, = 4·73 ,,

    C.   = 1·123   4·58

<div align="center">EXPERIMENT 30.</div>

Rabbit killed at 12.53 P.M. on 23.1.93.   Liver cut up.

A weighed 6·2 grms. (analysis lost).

B   „   7·3 grms.

C   „   7·2 „

D   „   8·0 „

Each placed in 150 cub. centims. of 0·75 per cent. salt solution at 37° C.   The salt solution of B had been previously sterilized.   Through D a stream of hydrogen was passed for ten minutes, and the tubes were then sealed.   All the bottles were placed in the incubator till 5 P.M. (4 hours) and then boiled.

<div align="center">

B.   Weight of glycogen = 0·273 grm. = 3·73 per cent.

C.   „   „   = 0·26   „   = 3·61   „

D.   „   „   = 0·296 „   = 3·70   „

</div>

The idea that the increased conversion of glycogen under the influence of chloroform is due to an impeded oxidation in the liver is, therefore, without experimental basis.

A digestive action of chloroform on proteids has been described by DENYS and MARBAIS ("Sur les peptonisations provoquées par le chloroforme." 'La Cellule,' vol. 15, p. 197; 'Nouvelles recherches sur la digestion chloroformique,' *ibid.*, I., 16, p. 4.)   These experiments tend to show that the hæmoglobin and the fibrin of dog's blood, whether in the serum of that animal, or in chloride of sodium solution of about 7 per cent. upon the addition of chloroform, ether, alcohol, thymol, or phenol, undergo a process of peptonisation.   They conclude, " La digestion chloroformique peut s'expliquer de deux façons ou bien par une action directe du chloroforme, ou bien par une zymase développée par ce dernier.   C'est la première hypothèse qui paraît la plus probable."

A study of the experiments on the influence of temperature upon this process tends, however, to the view that it is not a simple direct action of chloroform.   The digestion of entire blood, or of the hæmoglobin in it, is retarded by a temperature of even 45° C., and arrested by a temperature of 60° C., while the digestion of fibrin, which had not been subjected to a high temperature in serum previously boiled or deprived of its proteid by heating, does not exclude a possible action upon some substance *held in the fibrin.*

Perhaps the most inexplicable part of these researches, on the view adopted by them, is, that dog's fibrin is not digested in the serum of the ox, sheep, pig, or horse, on the addition of chloroform, *unless the serum has been previously boiled—i.e.,* unless it has ceased to be the characteristic serum.   The whole series of experiments, though interesting, are highly inconclusive, and throw little light upon the present series of observations.

The above experiments, especially when taken in connection with the observations on the influence of chloroform on the morphological changes in the liver cells

(see p. 248), seem to indicate that the *chloroform acts by accelerating the katabolic changes which accompany or immediately precede the death of the cells, and that it is as the result of these katabolic changes that we find the enormous conversion of glycogen.* That chloroform has this action in increasing katabolic changes is borne out by its influence on the disintegration of proteids. TANIGUTI (' Virchow's Archiv,' vol. 120, p. 121), after giving a *résumé* of the previous work on this subject, records his own experiments, which clearly show that chloroform does increase the excretion of nitrogen.

This view receives support from the mode of action of other agents on the liver cells, and on hepatic amylolysis.

4. *Ether.*

Although I am not aware that the administration of ether has been observed to be followed by glycosuria, the similarity of its action to that of chloroform seemed to render it desirable that its influence on hepatic amylolysis should be tested. The following experiments were accordingly performed.

EXPERIMENT 31. 17.11.91.

Rabbit killed at 2.5 P.M. Liver minced and divided into three parts.
A weighed 16·4 grms. placed in boiling water.
B „ 27·2 „ 0·75 per cent. NaCl solution with stream of air.
C „ 25·7 „ „ „ „ „ ether vapour.
B and C kept at 40° C. till 4.25 (2 hours 20 minutes).
Glycogen extracted by BRÜCKE's method.

$$A = 7·88 \text{ per cent.}$$
$$B = 6·40 \text{ „}$$
$$C = 5·87 \text{ „}$$

EXPERIMENT 32. 5.1.93.

Rabbit killed at 12.54 P.M. Liver minced and divided into three parts.
A weighed 10·0 grms. placed in boiling water at once.
B „ 11·9 „ „ 0·75 per cent. solution of NaCl with stream of ether.
C „ 10·2 „ „ „ „ „ „ air.
B and C kept at 40° C. till 9 P.M. (eight hours).
Glycogen extracted by BRÜCKE's method.

$$A. \text{ Glycogen} = 0·371 \text{ grm.} = 3·71 \text{ per cent.}$$
$$B. \quad „ \quad = 0·118 \text{ „} = 0·991 \text{ „}$$
$$C. \quad „ \quad = 0·271 \text{ „} = 2·650 \text{ „}$$

Ether, like chloroform, increases the amylolysis, but its action is much less decided. Like chloroform, it causes the early development of the cell changes described on p. 248 ; but in this respect, too, it is less powerful than chloroform.

5. *Pyrogallic Acid and Salicylate of Soda.*

The well-known influence of pyrogallic acid in accelerating the proteid waste and increasing the output of nitrogen (NOËL PATON, 'Brit. Med. Jour.,' 1886), and its marked destructive influence on hæmocytes, led me to investigate whether its action on hepatic amylolysis and on the liver cells corresponds to that of chloroform.

Salicylic acid, which in some of its actions resembles pyrogallic acid, was tried at the same time.

EXPERIMENT 33.   12.6.93.

Rabbit killed at 1.10 P.M.   Liver divided into four, A, B, C, D.
A weighing 5 grms., was placed in boiling water at 1.11.
B   „   10   „   „   „   0·25 per cent. pyrogallic acid dissolved in 0·75 per cent. NaCl.
C   „   8·5 „   „   „   0·75   „   NaCl.
D   „   10   „   „   „   0·5   „   salicylate of soda in 0·75 per cent. NaCl.
B, C, and D, kept at 40° C. till 2.30, then boiled.
Glycogen by BRÜCKE's method.

A   Glycogen = 0·185 grm. = 3·70 per cent.
B   „   = 0·165 „   = 1·65   „
C   „   = 0·157 „   = 1·84   „
D   „   = 0·216 „   = 2·16   „

Pyrogallic acid thus slightly increases hepatic amylolysis and at the same time it produces a slight acceleration of the changes in the protoplasm similar to those produced by chloroform.   Salicylate of soda in 0·5 per cent. solution does not accelerate these changes, nor does it increase hepatic amylolysis.

Among the substances the administration of which causes glycosuria are morphin, nitrite of amyl, and curare.

The following experiments were made on the influence of these bodies on the amylolysis of the excised liver :—

6. *Morphin.*

EXPERIMENT 34.   26.4.92.

Rabbit killed at 11 A.M.   Liver minced and divided into parts A, B, and C.
A weighing 22·5 grms. was placed in boiling water at 11.3 A.M.
B   „   23·0   „   „   about 150 cub. centims. of 0·75 per cent. NaCl in which 10 mgs. of bisuccinate of morphia was dissolved.
C weighing 21·8 grms. was placed in 0·75 per cent. NaCl.
B and C were kept at from 37° to 40° C. till 5 P.M. (6 hours).
Glycogen extracted by BRÜCKE's method.

A Glycogen weighed 0·861 grm. = 3·826 per cent.
B   „   „   0·310 „   = 1·35   „
C   „   „   0·369 „   = 1·69   „

EXPERIMENT 35. 16.5.92.

Rabbit killed at 11 A.M. Liver minced and divided into parts A, B, and C.
A weighing 16 grms. was placed at once in boiling water.
B   „   15·6   „   „   in 0·75 per cent. NaCl in which was dissolved 50 mgs. of hydro-
chlorate of morphia.
C weighing 17·0 grms. was placed in 0·75 per cent. NaCl.
B and C kept at 40° C. till 4 P.M. (5 hours).
Glycogen extracted by BRÜCKE's method.

A Glycogen weighed 0·518 grm. = 3·231 per cent.
B   „   „   0·211 „   = 1·352   „
C   „   „   0·202 „   = 1·188   „

Morphia has no influence on hepatic amylolysis in the excised liver.   Experiments 24 and 25 tend to show that it is also without action in the living animal.

7. *Nitrite of Amyl.*

With nitrite of amyl only one experiment was performed.

EXPERIMENT 36. 24.8.92.

Rabbit killed at 11.10 A.M. Liver divided into three parts A, B, C.
A weighing 14·5 grms. placed in boiling water at 11.12.
B   „   12   „   was placed in 0·75 per cent. NaCl. at 40° C.
C   „   12·5 „   „   „   „   „   „   „
A stream of air was passed through B and C, that through C being saturated with nitrite of amyl.
At 3.15 both were boiled.
Glycogen by BRÜCKE's method.

A Glycogen = 1·324 per cent.
B   „   = 0·720   „
C   „   = 0·704   „

Nitrite of amyl is therefore also without action on hepatic amylolysis.

8. *Curare.*

With curare a single experiment also was performed.

2 M 2

A very large, fat rabbit killed at 11.25 A.M.   Three portions of liver taken.

A weighing 21 grms. was placed in boiling water at 11.28.

B   „   26   „   „   0·75 per cent. NaCl.

C   „   25   „   „   about 150 cub. centim. of 0·75 per cent. NaCl. containing about
2 mgs. of curare which was known to be active.

B and C kept at 35° C. till 5 P.M. (5¼ hours), then boiled.

Glycogen extracted by BRÜCKE's method.

$$
\begin{array}{llll}
\text{A} & \text{Glycogen weighed } 3\cdot42 & \text{grms.} = 16\cdot29 \text{ per cent.*} \\
\text{B} & \text{„} & \text{„} & 3\cdot313 & \text{„} & = 12\cdot74 & \text{„} \\
\text{C} & \text{„} & \text{„} & 3\cdot315 & \text{„} & = 13\cdot26 & \text{„}
\end{array}
$$

From these observations it would seem that *while chloroform and ether increase
the amylolysis and thus produce glycæmia, nitrite of amyl, morphin, and curare act
in some other way, probably, as suggested by* ARAKI, *by diminishing the oxidation
changes in the tissues.*

In connection with this it may be mentioned that none of these bodies produce the
rapid changes in the protoplasm of this liver cell which are produced by chloroform
and ether.

II. IS THERE ANY DIFFERENCE IN THE PRODUCT OF HEPATIC AMYLOLYSIS
IN THE EARLY AND IN THE LATER PERIODS?

NATURE OF THE PRODUCTS OF AMYLOLYSIS IN THE EARLY AND IN THE
LATER PERIODS.

Upon the nature of the sugar found in the *post-mortem* liver, several investigations
have been made.

NASSE ('Pflüger's Archiv,' vol. 14, p. 473) describes the sugar of the liver as one
which, when boiled with sulphuric acid, acquires no increase in its reducing power,
and thus resembles glucose.

MUSCULUS and VON MERING ('Ztsch. f. phys. Chem.,' vol. 2, p. 416) confirmed
NASSE's observations. They, however, found maltose. Dextrin, however, was not
discovered. The method they adopted was to extract the liver with water, evaporate
the extract, and extract with alcohol, and then precipitate with ether.

SEEGEN ('Studien ü. d. Stoffwechsel,' p. 392) points out that the method of
MUSCULUS and MERING does not exclude certain dextrins which are precipitated with
ether, and gives experiments which tend to show that the maltose of these investiga-
tions is merely a mixture of dextrin and glucose. By dialysis he separates a sugar

* This is the largest amount of glycogen I have ever found in the liver of a rabbit.

which gave the reaction of glucose, and he concludes " dass Leberzucker aus-schliesslich Traubenzucker ist."

In a previous paper (*loc. cit.*, p. 383) he, however, says : " Wenn das Dialysat auf eine kleine Menge eingeengt und jetzt soviel absoluter Alcohol zugefügt war, bis die ganze Flüssigkeit einen ca. 90 proc. Alcohol bildete, entstand ein reicher weisser Niederschlag," and it is therefore somewhat surprising to find him using this method to differentiate dextrin from maltose. Musculus and Meyer ('Bull. Soc. Chim.,' vol. 35, p. 370) also state that dextrin is slightly diffusible.

Limpricht ('Liebig's Annalen,' 133–293, quoted by Nasse) extracted from 200 lbs. of the liver of the horse as much as 400 grms. of dextrin.

Külz (Pflüger's 'Arch.,' vol. 24, p. 52) severely criticizes Seegen's paper, and confirms Nasse's results. He says : "Ob Dextrin und Maltose in der todtenstarren Hundeleber vorkommen oder fehlen, wage ich vorläufig mit Sicherheit nach keiner Seite hin eine Behauptung auszusprechen, bemerke jedoch dass für mich nur die Darstellung dieser Körper in Substanz beweisskräftig ist."

Musculus and von Mering ('Ztsch. f. phys. Chem.,' vol. 4, p. 93) attack Seegen's results, and show that his methods are unsatisfactory, though they do not give any experimental evidence in support of their assertion as to the presence of maltose, nor attempt to answer directly Seegen's criticism on their methods.

The question stands thus :—All investigators admit that the chief sugar of the excised liver is glucose. Limpricht has definitely shown that dextrin may be present. Musculus and von Mering hold that maltose is also to be found.

A general consideration of the relationships of the lower dextrins to such di-saccharids as maltose clearly shows that a complete separation is most difficult, if not impossible. Many dextrins are partly soluble even in alcohol, much over 90 per cent. ; maltose is less soluble in strong alcohol than glucose ; dextrin in alcoholic solution, as well as maltose, is precipitated by ether, and forms a similar potash com-bination ; the osazone of dextrin is soluble in water, while that of maltose is only *somewhat* less soluble ; lastly, dextrin, though less diffusible than maltose, does undoubtedly pass through a dialyzing membrane.

For these reasons, after several unsuccessful attempts, the endeavour to separate the lower dextrins from maltose was abandoned, and attention was concentrated on the question of whether, along with glucose, dextrin, and maltose, or one or other of these, are produced at all periods in the liver excised from the body.

Method.

The liver was extracted with boiling water exactly as in the estimation of glycogen ; the hot aqueous extract was then precipitated with chloride of iron and acetate of soda, carbonate of soda being added to the point of neutralization. In this way, not only the proteids, but also glycogen, as shown by Landwehr (' Ztsch. f. phys. Chem.,' vol. 8) are thrown down. The clear supernatant fluid containing the sugars and

dextrins (LANDWEHR, *loc. cit.*, p. 170*) was filtered off and, 1st, tested for dextrin, by the addition of absolute alcohol, to 90 per cent.; 2nd, tested for sugar as glucose, by FEHLING's solution; and, 3rd, a given volume was boiled for some time with 2 per cent. H_2SO_4, then neutralized, made up to its original volume, and the glucose again estimated with FEHLING's solution.

To determine if the process is the same, during the rapid initial and during the slow later amylolysis, livers kept in 0·75 per cent. salt solution at from 37 to 40° C. were tested during the first hour, and, at the end of eight or ten hours in the above manner :—

A marked difference was found.

During the first hour :—

No precipitate was given with alcohol; dextrins were absent.

The reduction was exactly the same before and after boiling with H_2SO_4.

After several hours :—

There was usually a marked precipitate with alcohol, though no reaction with iodine. An achroö-dextrin, but no erythro-dextrin, was present. The reduction was usually markedly increased after boiling with H_2SO_4. Dextrin and maltose, or dextrin alone, were present.

The increase in the reducing power after boiling with sulphuric acid is usually so great that it cannot be due to a conversion of maltose to glucose, but must arise from the change of dextrin to glucose.

This is well shown in Experiment 40 which was performed to elucidate the influence of the acid in the liver. One part of the liver had been digested for ten hours with a weak alkali, carbonate of soda 0·06 per cent., while the other part was kept in salt solution and the acid allowed to develop. In the former, the reduction before boiling was equivalent to that produced by 2·8 per cent. of glucose; after boiling with H_2SO_4, it was equal to 3·2 per cent. In the latter, before boiling, the reduction was equivalent to that produced by 2·0 per cent. glucose, after treatment with an acid to 2·3 per cent. glucose.

These observations show that during the early amylolysis the product is glucose, during the later amylolysis intermediate products such as dextrin and possibly maltose also are formed.

III. NATURE OF HEPATIC AMYLOLYSIS.

We are now in a position to consider the nature of the early and of the later amylolysis in the excised liver.

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* NASSE (PFLÜGER's 'Archiv,' vol. 37, p. 582) says that achroö-dextrin is precipitated with iron; but his objections are met by LANDWEHR (PFLÜGER's 'Archiv,' vol. 38, p. 321). My own observations on erythro-dextrin and the achroö-dextrin formed from glycogen confirm LANDWEHR's results.

A. EARLY AMYLOLYSIS.

Is the early, rapid change the result of the action of a ferment, or is it due to changes in the liver cells ?

The *rapidity* with which it occurs is entirely opposed to the idea of its being due to the amylolytic ferment which may be extracted from the liver as from other organs, and which, according to all observers, acts very slowly.

The fact that the mechanical destruction of the liver-cells so markedly inhibits the process is also strongly opposed to the idea that the essential agent is a soluble ferment.

The connection of the process with the condition of the liver cells favours the view that the activity of the cells is the agent causing the amylolysis.

The influence of temperature and of fluoride of sodium, the increased amylolysis under chloroform, ether, and pyrogallic acid which accelerate the katabolic changes in the liver cells, and the absence of increased amylolysis with morphin, curare, nitrite of amyl, and salicylate of soda, seem to point to intra-cellular changes being the important factor in this early period.

Finally, the direct production of glucose, without intermedial bodies, marks off the changes at this period from those occurring later.

This early change in the excised liver is simply a continuation of the vital process in the organ, though the katabolic side of the metabolism is exaggerated, and the anabolic in abeyance.

In fact, all the evidence is in favour of the view that *the conversion of glycogen to glucose is precisely the same as the conversion of mucinogen to mucin or of zymogens to zymin. And there is no more reason to invoke the agency of a ferment in the explanation of the former than of the latter processes.*

B. LATER AMYLOLYSIS.

But when we come to consider the later amylolytic changes, which do undoubtedly go on in the liver after the death and destruction of the cells, the question becomes more involved. Is the change due to the *acid* which makes its appearance, to the agency of *micro-organisms,* or to the development of a soluble ferment, or *zymin ?*

1. *Action of Acid.*

As has been shown by SEEGEN and other observers, the post-mortem liver becomes more and more markedly acid. SEEGEN (*loc. cit.,* p. 40) shows that lactic acid is present.

This acidity is largely due to the action of micro-organisms, as is shown by the following experiment.

<div align="center">EXPERIMENT 38.</div>

The liver of a rabbit freshly killed was excised and divided up, the instruments and hands having been washed in methylated spirit, and every precaution to prevent ingress of organisms taken.

A was placed in 0·75 per cent. salt solution.

B   ,,   salt solution, with chloroform.

C   ,,   salt solution, previously sterilized.

All were kept at 40° C. for eight hours.

At the end of this—

A was markedly acid.

B was neutral.

C   ,,

Gelatine tubes inoculated from—

A showed in twelve hours a most active growth of a liquefying organism.

B showed no growth after two weeks. .

C showed a growth after three days of an organism resembling Bacillus subtilis in its characters, but forming a thicker and more wrinkled pellicle.

SEEGEN demonstrated that lactic acid, when boiled with glycogen, converts it to dextrin, but DASTRE (*loc. cit.*, p. 93) finds that glycogen in presence of lactic acid at 40° C. for twelve to sixteen hours is not changed to sugar.

I find that glycogen may be kept in the presence of lactic acid, of considerably greater strength than is found in the post-mortem liver, for two days without undergoing any change.

On the third day a slight reduction with FEHLING may occur. Tubes inoculated from the flask gave no growth of micro-organisms, so the action could only have been due to the acid. If the acid is boiled with glycogen, a similar slight reduction is got after twelve hours. .

From these facts it seems highly improbable that the presence of the organic acids can have any action in the late hepatic amylolysis. To determine this point the following experiment was performed.

<div align="center">EXPERIMENT 39.   10.5.93.</div>

A rabbit, which had on the previous day thrown a litter of young, was killed at 11.35 A.M. In excising the liver the animal's skin, the hands, instruments, scale pan, &c., were well washed with perchloride of mercury, and then with methylated spirit. The liver was not allowed to come in contact with the skin of the animal or with any body not sterilized. The flasks into which the organs were placed, had, with their contents, been previously sterilized.

A weighing 7·1 grms. was placed in boiling water at 11.37.

B   ,,   8·5   ,,   ,,   about 150 cub. centims. of 0·75 per cent. NaCl. ·

b   ,,   11·8.   ,,.   ,,.   ,,   ·   ,, ·   ,,   ,,   . to which carbonate of soda had been added to 0·06 per cent.

C weighing 11·0 grms. was placed in about 150 cub. centims. 0·75 per cent. NaCl. ·

c   ,,   12·5   ,,   ,,   ,,   ,,   ,,   ,,   to which carbonate of soda had been added to 0·06 per cent.

B and b were kept in the incubator at 40° C. till 1.20 P.M., about 1 hour.

C and c were kept at 40° C. till 9 P.M., about 8½ hours.

Before boiling, gelatine tubes were inoculated from each. All remained sterile except C, in which a slight growth of a coccus appeared after three days. C was markedly acid with a sour smell. c was neutral and devoid of putrefaction or a sour smell.

Glycogen was extracted by BRÜCKE's method.

A. Glycogen weighed 0·181 gram. = 2·54 per cent.
B   „   „   0·106   „   = 1·24   „
b   „   „   0·14   „   = 1·23   „
C   „   „   0·11   „   = 1·00   „
c   „   „   0·149   „   = 1·19   „

EXPERIMENT 40.   29.5.93.

Rabbit killed at 12.11, and the liver divided in the usual manner.

A weighing 6·9 grms., was placed in boiling water at 12.13.

B   „   12·0   „   „   „   at 12.15 in 0·75 per cent. NaCl to which carbonate of soda to 0·06 per cent. was added.

C weighing 11·6 grms. was placed in 0·75 per cent. NaCl at 12.15.

B and C were kept at 40° C. in the incubator till 9 P.M., and then boiled. B had a strong odour of liver, and was neutral. C had a marked sour putrefaction odour, and was strongly acid. Gelatine tubes were inoculated from each. C gave a strong growth of organisms (Bacillus subtilis) in two days.

B gave a slight growth in four days.

The watery extract of each part of the liver after evaporation was divided into equal parts.

From one part glycogen was extracted by BRÜCKE's method. In the second part the glucose was estimated in the usual manner.

To 2 cub. centims. of the solution after the separation of glycogen, absolute alcohol was added to 90 per cent.

A gave no precipitate.

B and C gave slight precipitate on standing, indicating the presence of dextrins.

None gave any reaction with iodine.

A. Glycogen weighed 0·147 = 4·3 per cent.
B   „   „   0·091 = 1·5   „
C   „   „   0·095 = 1·6   „

Sugar (as glucose) determined by FEHLING's solution. The suboxide precipitated very perfectly.

A 44·5 cub. centims. gave no complete reduction of 2 cub. centims. of FEHLING's solution.

Sugar as glucose less than 0·2 per cent.

B 5·8–6·0–5·8 = 5·8 cub. centims. gave complete reduction of 2 cub. centims. of FEHLING's solution.

Sugar as glucose = 2·8 per cent.

C 12·8–12·4 = 12·6 cub. centims. gave complete reduction of 2 cub. centims. of FEHLING's solution.

Sugar as glucose = 2·0 per cent.

To 40 cub. centims. of B and 50 cub. centims. of C a few drops of H_3SO_4 were added in flasks which were boiled in the water bath for $2\frac{1}{2}$ hours. The contents of each flask were then neutralized and made up to the original volume, and the glucose estimated by FEHLING's method.

Both gave a very perfect precipitation of the suboxide.

B 4·8–5·0–5·0 (5·0 cub. centims.) completely reduced 2 cub. centims. of FEHLING's solution.

<div align="center">Glucose = 3·2 per cent.</div>

C 10·7–10·8 (10·8 cub. centims.) completely reduced 2 cub. centims. of FEHLING's solution.

<div align="center">Glucose = 2·3 per cent.</div>

	Glycogen.	Sugar as glucose.	Glucose, after boiling with acid.
	per cent.	per cent.	
A	4·3	less than 0·2	—
B	1·5	2·8	3·2
C	1·6	2·0	2·3

The smaller amount of sugar in C is probably due to the action of micro-organisms in destroying it.

These two experiments very clearly show that both *the early and the later amylolysis are independent of the development of an acid in the liver.*

The latter further shows that the peculiar indirect amylolysis of the later period proceeds in the same manner whether the acid be allowed to develop or not.

2. *Action of Micro-organisms.*

DASTRE (*loc. cit.*) maintains that these *post mortem* changes are not due to a soluble ferment derived from the liver-tissue, but simply to the action of micro-organisms. He says : "Les fermentations glycosiques que l'on a obtenues avec la macération ou la décoction du foie sont le résultat de l'activité des microbes," and supports this statement by experiments to show that, not only after boiling and heating to 110° C. does the tissue lose all power of converting glycogen, but that, after sterilizing at 55° C.—a temperature which he concludes does not injure a soluble ferment—all amylolytic power is lost.

Undoubtedly, certain microbes have a slow amylolytic action. Several organisms change glycogen, in part at least, to a reducing sugar when kept at 40° C. for twelve hours.

The differentiation of those which act and those which do not act forms no part of the present inquiry. That they are the amylolytic agents in the liver is opposed by the careful experiments of EVES on the isolated ferment, and by the following experiments.

<center>EXPERIMENT 41. 23.1.93.</center>

Rabbit killed at 12.57 p.m. Liver cut into pieces A, B, and C.

A weighed 6·2 grms. placed in boiling water at 1 p.m.

B   ,,   7·3 grms.   ,,   0·75 per cent. NaCl solution unsterilized.

C weighed 7·2 grms. placed in 0·75 per cent. NaCl solution sterilized.

B and C kept at 50° C till 5 p.m. (4 hours).

Glucose estimated as in previous experiments.

<blockquote>
Filtrate of A = 0·037 grm. glucose = 0·596 per cent. glucose in liver.

  ,,   B = 0·27   ,,   ,,   = 3·69   ,,   in liver.

  ,,   C = 0·26   ,,   ,,   = 3·61   ,,   ,,
</blockquote>

Glucose formed per 10 minutes from commencement.

<blockquote>
B = 0·125 per 100 parts of liver.

C =   ,,   ,,   ,,
</blockquote>

Cultures on glycerine agar from B gave copious growth, from C gave no growth.

<center>EXPERIMENT 42. 13.1.93.</center>

Rabbit killed at 12.58 P.M. Liver minced and divided into A, B, and C.

A weighed 10·6 grms. placed at once in boiling water at 1 P.M.

B   ,,   11·4   ,,   ,,   in sterilized solution of NaCl, 0·75 per cent.

C   ,,   11·5   ,,   ,,   non-sterilized solution of NaCl, 0·75 per cent.

B and C kept at 40° C. till 4.15 (4 hours 15 minutes).

Glycogen extracted by Külz's method.

<blockquote>
A   Glycogen = 1·164 grms. = 10·981 per cent.

B   ,,   = 1·034   ,,   =   9·07   ,,

C   ,,   = 1·109   ,,   =   9·64   ,,
</blockquote>

Glycogen lost per 10 minutes from commencement.

<blockquote>
B   0·051 per cent.

C   0·075   ,,
</blockquote>

Cultures on agar-agar from B gave no growth, from C a copious growth of micro-organisms (see also Experiment 30, p. 264).

In addition to these we may refer to Experiments 13 and 17 on the influence of chloroform, an agent preventing the growth of organisms, on the late amylolysis. Such experiments seem to *exclude the action of micro-organisms as the main factors in this late amylolysis.*

<center>2 N 2</center>

3. *Action of Zymin.*

The action of both the acids and of micro-organisms having thus been excluded, and the existence of a true ferment having been demonstrated in the liver some time after death, we are forced to the conclusion that *it is by the action of a ferment, in some cases assisted by the influence of micro-organisms, that the amylolosis which proceeds in the liver for so long after death is carried on.*

I have made no definite series of experiments upon this ferment, but I have satisfied myself that it can be extracted from the liver, and that it acts very slowly and incompletely. The researches of Eves* and others render further work upon it needless.

When the production of sugar, the result of the intracellular or vital changes, ceases, and when the ferment action begins, it is impossible to say. Almost certainly the two processes greatly overlap one another. Nevertheless, they are of totally different nature and should be carefully distinguished.

The general results of these investigations may be summarized :—

1st. The great and active disappearance of glycogen in the excised liver, kept at the body temperature, is during the first half hour. The rate of conversion steadily diminishes during the remainder of the first hour, and after two hours goes on very slowly (Experiments 1 to 6).

2nd. An enormous diminution in the amylolysis is produced by destroying the structural integrity of the liver cells (Experiments 7 and 8).

3rd. The active early amylolysis goes on before and during the development of structural changes in the liver cell, while the slower amylolysis advances after the cell structure is completely destroyed.

4th. Exposure for an hour to a temperature of 60° C. greatly retards, but does not arrest, amylolysis (Experiments 9 and 10).

5th. Fluoride of sodium in 1 per cent. solution greatly retards or stops the early amylolysis, but does not arrest the later changes (Experiments 11 and 12). It does not accelerate the structural changes in the liver cells.

6th. In the excised liver the amylolysis is enormously increased in rate in the presence of chloroform (Experiments 13 to 17).

7th. In this amylolysis the glycogen is changed to glucose (Experiment 18).

8th. It is the early active amylolysis, and not the later slower conversion, which is accelerated by chloroform (Experiments 19 to 21).

9th. The structural changes in the cells are greatly hastened and increased under the influence of chloroform.

10th. In the living animal, as in the excised liver, chloroform hastens the conversion of glycogen to glucose (Experiments 24 to 28); and the glycæmia and glycosuria of chloroform poisoning are probably due to this.

* *Loc. cit.*

11th. The accelerated conversion is not due to diminished oxidation (Experiments 29 and 30), but, apparently, to a direct action on the liver cells hastening the katabolic changes.

12th. Ether has a similar, but less marked, action to chloroform (Experiments 31 and 32).

13th. Pyrogallic acid acts in the same manner (Experiment 33).

14th. Morphin (Experiments 34 and 35), curare (Experiment 37), nitrite of amyl (Experiment 36), and salicylate of soda (Experiment 33), neither hasten the structural changes in the liver cells nor modify the rate of amylolysis. The glycosuria caused by the first three of these substances is, therefore, not due to accelerated hepatic amylolysis.

15th. During the early amylolysis the product is glucose; during the later changes intermediate bodies, dextrins, and possibly maltose, are also formed.

16th. The early changes in the excised liver are simply a continuation of the vital processes in the organ—the katabolic side of the metabolism being exaggerated, the anabolic side in abeyance.

17th. The change of glycogen to glucose is thus precisely analogous to the change of mucinogen to mucin and of zymogens to zymins, and is dependent on the metabolism of the cell protoplasm, and not upon the action of a zymin.

18th. The later amylolytic changes in the excised liver are not due to the development of acid (Experiments 39 and 40), nor are they largely due to the action of micro-organisms (Experiments 41 and 42 and 13 to 17), but they are caused by the development of a zymin probably formed in the disintegration of the cells.

NOTE, January 24, 1894.—It has been suggested to me by Mr. HORACE T. BROWN, F.R.S., that the slowing of the amylolysis during the first hour may be due not to the death of the cells but to the accumulation of the products of activity inhibiting the process. The following experiment seems to negative such an explanation.

Rabbit killed at 1.15 on 18.1.94. Liver divided into A, B, and C.

A weighing 4 grms., was placed in boiling water.

B   „   10·2 grms., was placed in 0·75 per cent. NaCl solution in a beaker.

C   „   11·4   „   „   „   „   „   vegetable parchment sausage paper suspended in a large quantity of salt solution and frequency agitated.

B and C were kept at 41° C. for 4 hours and then boiled.

The fluid around C, on evaporation, gave a reduction with FEHLING'S solution.

Glycogen by BRÜCKE'S method.

$$
\begin{array}{lllll}
\text{A} & \text{Glycogen weighed} & 0\cdot225 \text{ grm.} & = 5\cdot62 \text{ per cent.} \\
\text{B} & \text{„} & \text{„} & 0\cdot225 \text{ „} & = 2\cdot22 & \text{„} \\
\text{C} & \text{„} & \text{„} & 0\cdot280 \text{ „} & = 2\cdot45 & \text{„}
\end{array}
$$

VII. *On the Characters and Behaviour of the Wandering (Migrating) Cells of the Frog, especially in relation to Micro-Organisms.*

By A. A. KANTHACK, *M.R.C.P., M.D., Lecturer on Pathology, St. Bartholomew's Hospital, London; and* W. B. HARDY, *M.A., Fellow of Gonville and Caius College, and Junior Demonstrator of Physiology to the University of Cambridge.*

(*From the Pathological and Physiological Laboratories, Cambridge.*)

Communicated by Professor M. FOSTER, *Sec. R.S.*

[PLATE 29.]

INTRODUCTORY.

SECTION V.—The behaviour of the cells towards active growths of *Anthrax* and *B. fila-mentosus.*

 General description of the phenomena.

  Exp. i., with *Anthrax* $\begin{cases} a, \text{ cells winning.} \\ b, \text{ „ losing.} \end{cases}$

  „ ii., „ „ „ winning.

  „ iii., „ *B. filamentosus* „ losing.

 The hyaline cell unable to ingest the bacilli until they have been attacked by the eosinophile cells.

 Further evidence derived from :—

  (*a*) The behaviour of the cells towards bacilli and spores when both are present. Exp. iv.

  (*b*) Differential action of the cells under the influence of heat or urari, and in œdematous Frogs. Exp. v.

 The discharge and reformation of granules—the amphophile granulation of the eosinophile cell. Exp. vi.

SECTION VI.—Further discussion of the behaviour of the cells towards foreign substances. The arrangement of such substances in a series.

 Action of yeast. Yeast intermediate in its effects between substances treated of in Section IV. and those in Section V. Exp. vii.

 The two results of the action of the hyaline cells on foreign particles.

SECTION VII.—The rose-reacting cells. The action of foreign substances when solid, or when dissolved. The removal of the latter by (*a*) the rose-reacting cells, (*b*) by excretory organs.

 Observations on *Astacus* and *Daphnia* bearing on this.

SECTION VIII.—Preliminary observations on Mammals (Rabbits and Rats).

 Summary of the preceding sections.

SECTION IX.—Discussion of the morphological and physiological condition of the wandering cells.

  Morphology. A comparison of the structure of the sporadic mesoblast in different animals leads to the conclusion that the different cell-elements of higher forms have arisen by a process of differentiation from a simple amœboid cell which possessed all their characters and performed all their functions.

  Physiology. The primitive cell of generalized functions has differentiated into the granular eosinophile cell, the ingestive hyaline cell, and the absorptive rose-reacting cell. The relation between the phenomena exhibited by the eosinophile cell and the hyaline cell, illustrated by reference to parallel phenomena in the Protozoa and elsewhere.

 The question of immunity referred to.

INTRODUCTORY. (Added September, 1893.)

[The most salient feature of the wandering cells of the body is their marked increase in numbers in inflammation. VIRCHOW, originally, in his great work, 'The Establishment of the Cellular Pathology,' traced the "Rundzellen Infiltration" to a proliferation of connective tissue cells. Later, however, RECKLINGHAUSEN and COHNHEIM showed that the white cells of the blood could penetrate the walls of the

blood capillaries, and so pass to an inflamed area. Thus, the first step was taken towards defining the morphological position of the wandering cells as an independent portion of the body.

The fact that the wandering cells ingest discrete particles has long been known. CLAUS, SCHULTZE, HAECKEL, HOEK, and others described the process as occurring in both Vertebrates and Invertebrates. But the genius of METSCHNIKOFF[*] first suggested the importance of this process to the body. He showed that ingestion was, in the case of bacteria and digestible ingesta generally, followed by digestion. This discovery enabled METSCHNIKOFF to attain to some conception of the real significance of the inflammatory process, and the part the wandering cells play therein.

While METSCHNIKOFF was working at this question, startling additions were being made to our knowledge of the life-history of vegetable micro-organisms and the part they play in disease. The enormous importance of his discoveries, as suggesting an explanation of the resistance of animals to the invasion of these bodies, became at once obvious, and METSCHNIKOFF was led to formulate a theory of the immunity of animals in which the resistance to the disease germs is referred solely to the phagocytic activities of the wandering cells.

From this time onwards the wandering cells have been regarded almost solely from the relatively narrow standpoint of their relation to the conflict with pathogenic germs.

The next advance in our knowledge was the direct outcome of the labours of those bacteriologists who refused to accept the phagocytic theory of immunity as a complete solution of that problem.

The discussion of the origin of immunity, like the earlier discussion of the origin of pus corpuscles, led to the enunciation of extreme theories. Those observers who found themselves in opposition to METSCHNIKOFF, went so far as to deny to the wandering cells any direct participation in the processes which attend the development of immunity, and, without attempting to explain how the plasma gained such properties, they gave to the world one or other form of a "humoral theory."

In this way two distinct schools arose, one which attributed all to the phagocytic activity of the cells, another which regarded the fluid plasma as all important.

The result of an attempt to effect a compromise between these extreme views was the formation of a third school, which regards any special activity of the plasma as being due to substances derived from the cells, and its adherents find their support in the work of WOOLDRIDGE, of HANKIN, and of BUCHNER.

In order to see how this led to a wider appreciation of the functions of the wandering cells it is necessary to trace briefly the main lines of the discussion.

In 1887, FODOR, NISSEN, and others established the fact that the blood serum

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[*] 'Annales de l'Institut Pasteur,' 1887, pp. 321–336; *ibid.*, 1889, p. 25, *et. seq.*; *ibid.*, 1889, p. 289, *et. seq.*; *ibid.*, vol. 4, p. 65, *et. seq.*; *ibid.*, vol. 4, p. 465. VIRCHOW's 'Archiv,' vol. 97, p. 177; *ibid.*, vol. 107, p. 209; *ibid.*, vol. 109, p. 176; *ibid.*, vol. 113, p. 63.

possesses marked bactericidal powers, and the result of this discovery was the enunciation of a humoral, as opposed to a cellular theory of immunity.

METSCHNIKOFF, and other supporters of the cellular theory, soon proved that this bactericidal power was not possessed by blood in the body, but was developed as a result of *post mortem* changes, and, in 1888, BUCHNER clearly correlated the development of the bactericidal power with the breaking up of the leucocytes in shed blood, and, in 1890, HANKIN succeeded in isolating a bacteria-killing substance from lymphatic glands and the spleen. Thus, the idea that some, at any rate, of the wandering cells were concerned in the production of a peculiar substance characterized by its bactericidal power was suggested.

The nature of this substance, and especially the nature of its action on bacteria, whether, for instance, it destroys them or merely hinders their growth, are questions which have engaged the attention of many workers, but which do not concern us here.

Turning to the development of our histological knowledge of the wandering cells, we find that the existence of more than one kind of leucocyte in Vertebrates was recognized at a very early period. But it was not until EHRLICH,* in 1878, drew attention to the specific granulation of these bodies, and, with the help of the aniline dyes, provided us with a method of histo-chemical analysis, whereby the different forms could be readily recognized, that any great advance in the direction of determining differences of function became possible.

The striking histological advances made by EHRLICH and his school were, until quite lately, completely ignored in the discussion which raged around the cellular theory of immunity, and the wandering cells still continued to be spoken of indifferently as phagocytes, with no recognition of the possible existence of diverse forms endowed with diverse functions.

It is difficult to determine when or how the attention of those concerned in the discussion came to be attracted to the granulation of the cells, but it may be traced mainly to the work of BUCHNER, HANKIN, and those others who derived the bactericidal substance from the leucocytes.

Although the observations which we are about to record have, we venture to think, a direct bearing on the theory of immunity, we would ask that they might be regarded, not in this light only, but as some contribution to the more general natural history of the wandering cells.]

On investigating the histology of the body fluids of certain Invertebrates and Vertebrates, we find that animals widely separated in structure and habits possess the same kinds of wandering cells. But we also find (1) that, within the limits of a single group of animals, the simplest forms possess only one kind of wandering cell, while those with greater structural complexity have all the three typical forms sharply and completely distinct from one another ; and (2) that during the fœtal

* EHRLICH, ' Farbenanalytische Unters.,' Berlin.

period a Mammal has only one kind of wandering cell. These facts suggest two ideas : firstly, that a certain fixity of type must be accorded to each kind of wandering cell, that the different forms found in the more complex animals must be regarded as distinct from one another in their development and life history, even if they be regarded as having a common origin ; and, secondly, that, corresponding with this divergence and fixity of type, there must be divergence and fixity of function. We have endeavoured to demonstrate a disparity of function comparable to the disparity of form by comparing the behaviour of the different kinds of wandering cells towards various substances when added to the lymph or blood.

Since the wandering cells of the Frog retain their vitality for a long time after removal from the body, that animal has been mostly used. The lymph is obviously most serviceable for examination, but the cells of the blood are similar and offer similar phenomena.

Some observations have also been made on *Astacus*, the Rat, and the Rabbit.

The experiments on the cells when out of the body have been controlled by parallel experiments on the cells while still within the body.

We have to thank many friends for willing help and criticism, but special acknowledgment is due to Miss GREENWOOD for allowing us to see her preparations, and follow the results of her later and still unpublished work on digestion in Protozoa.

SECTION I.

Methods Employed.

In determining the identity of the wandering cells, we have had regard to differences as to shape, whether the cell be resting or amœboid, as to the texture of the cell-substance, as to the nuclear type, and as to the presence or absence and the histo-chemical nature of the cell-granules. The shape and appearance of the cells when resting is not more serviceable for their identification than their appearance when active. The manner of emitting pseudopodia, and the appearance of the cell when it has thrust out these appendages is markedly different in different cells.

Differences in the texture of cell-substance are brought into marked prominence by the use of iodine, and this reagent cannot be too highly praised in this connection.

The nuclear type of the various cells has been studied with the aid of a solution of methyl-green, slightly acidulated with acetic acid, and to which a trace of osmic acid has been added.

Nuclear characters are also shown by treatment with an alkaline alcohol-osmic acid solution of methylene-blue, which is practically LOEFFLER's solution with much less methylene-blue present and with a trace of osmic acid added. With this solution eosinophile* granules remain entirely uncoloured and unchanged. Amphophile

* EHRLICH, ' Farbenanalytische Unters.,' Berlin.

granules are stained blue, or rarely a very dull violet when viewed with yellow light. And the basophile granules appear violet with daylight, and brilliant rose with yellow light. Nuclei and microbes are blue with both lights. The substance which produces the rose-coloured modification of methylene-blue does so whether it be present as granules in the cell-substance, or dissolved in the surrounding fluid. The reaction also survives with unaltered intensity when the preparation is dried at the temperature of the air and mounted in Canada balsam.

The study of the living cells, and their behaviour towards noxious or innocuous substances has been carried out (1) by injecting various substances into the lymph spaces of the Frog, and withdrawing drops of lymph for examination at varying intervals of time, and (2) by hanging drops. The hanging drops were suspended on the under side of a cover-slip in moist chambers sufficiently large to provide air enough for the needs of a small drop of lymph for about ten hours, without introducing a fresh supply. In this simple way we have been enabled to continuously observe the processes taking place in a drop of lymph or blood after inoculation with microbes, poisons, &c., for periods up to ten hours. Discontinuous observation has been kept up for 40 to 50 hours. The cover-slips used were always carefully cleaned with acid and absolute alcohol, and then sterilised by heat immediately before use.

The study of these drop-cultures was controlled by examining lymph taken from the lymph sac and peritoneal cavity of a Frog, into which microbes, &c., had been injected. In all cases the most complete accord was found, frequently extending to the element of time.

To study the effects of temperature, the drops were placed on the ordinary metal stage, through which warmed or cooled water was circulating. In these experiments it is essential that the whole chamber and the cover-slip should be brought to the requisite temperature before the lymph is added. Otherwise the earlier stages will occur before the temperature has either risen or fallen to the required point.

It is most important to note that, in order to obtain satisfactory results, it is necessary to use only freshly captured Frogs.

SECTION II.

Histology of the Frog's Wandering Cells.

In the body of the Frog three kinds of wandering cells occur. These are (1) the eosinophile cell, (2) the hyaline cell or phagocyte, and (3) the basophile cell with rose-reacting granules. These, together with the red corpuscles and platelets, constitute the sporadic mesoblast of the Frog, thus constituting a tissue whose elements, unlike the elements of the other tissues, have no coherence, and but little fixity of place. Like the other tissues of the body, however, this particular tissue increases or decreases in bulk in correspondence with certain bodily needs, the

increase in bulk being largely due to the multiplication of the cells, whether eosinophile, hyaline, or rose-staining, by binary fission. This occurs freely in the body fluids, and may be watched outside of the body in a hanging drop.

(1.) The *eosinophile cell* (Plate 29, figs. 1–3, 10, 14), when resting, is spherical in shape. The more central portion of the cell is occupied by a greater or less number of highly refractive granules, each granule having a yellow-green lustre. These granules were identified by EHRLICH with his α, or eosinophile group.* With very high magnification the individual granules sometimes appear to have a short and long axis, and to be slightly spindle-shaped. The central portion of the cell, or endosarc, which contains the granules and the nucleus, is clearly distinguished from the delicate ectosarcal layer of very transparent mobile protoplasm, though, in the Frog, owing to the smaller size of the cell, the distinction is not so beautifully shown as it is in the larger eosinophile cell of *Astacus*.† The nucleus of the eosinophile cell is exceedingly characteristic. It is elongated and bent to a horse-shoe shape. The chromatin filaments are either irregular or radiate from two nodes, situated towards either end of the nucleus. Sometimes the nucleus is trilobed. When proliferation of the eosinophile cells is taking place they are to be found with more than one nucleus. Under appropriate stimuli the eosinophile cell becomes very active. Such stimuli are, normally, of a chemical nature and may be regarded as a change in the surrounding fluid. This may be produced either by clotting, or by the introduction of foreign substances, such as microbic poisons. If the cell is floating freely in the fluid, then the activity is confined to the thrusting out of the ectosarc as short filiform pseudopodia, which radiate from the still spherical endosarc, and do not necessarily result in locomotion. If, however, the cell is in the neighbourhood of a chain of active microbes, then the pseudopodial activity becomes so far modified, that the cell progresses towards the chain. Lastly, when the cell effects actual contact with the microbe the pseudopodial activity becomes suddenly changed into violent streaming movements, which result in the extension of the cell along the chain (figs. 10, 14, 17). So-called indifferent particles of any kind or shape, such as Indian ink, in no wise affect the activities of the cell, even though accidental contact occur. Contact with an active microbe, however, not only stimulates the cell to increased movement, but also produces a new activity of a glandular nature. The granules are thrust from the endosarc into the ectosarc, and travel towards that portion of the cell which is in contact with the chains; there they rapidly lose their brilliant refractive nature, shrink in size and disappear. (Fig. 10B.)

Complete discharge of the granules may occur, and then after an interval the cell may reform its granules which, at first, are different from the initial eosinophile granule. These processes are described in detail in a later section; enough has been

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* EHRLICH, "Ueber die specifischen Granulationen des Blutes," 'Verhand. d. Physiol. Gesellsch. zu Berlin,' 1878.

† Compare figs. 1 and 5, Plate 7, 'Journal of Physiology,' vol. 13.

said here to substantiate the statement that in the eosinophile cell we are dealing with one which has both glanular and amœboid properties corresponding to differentiation of the cell's body into a central glandular endosarc, and a peripheral contractile ectosarc.

A change of medium also stimulates cell division. This process we have watched in a drop culture (Exp. 1), and in all cases the daughter-cell has been free from granules. In a comparatively short time granules commence to be formed at a point situated rather to one side of the centre of the daughter-cell.

The Re-formation of Granules.

The Amphophile Granule (fig. 17).—The glandular activity of the cell does not always result in a complete obliteration of the granule as a histological feature of the cell. Under the influence of poisons, *e.g.*, urari, or bacterial products, the granules may be so far altered that they stain with methylene-blue as well as with eosin. In other words they become amphophile in the sense in which EHRLICH uses the term. The relation of this change to the complete discharge of the granule can be better discussed after the different phenomena exhibited by the eosinophile cell have been more fully described. The amphophile granule is also produced in another way. The eosinophile cell will, after the complete or partial discharge of its granules, load itself with newly formed ones. These at first appear to be always amphophile, and there is, therefore, a stage in the elaboration of the eosinophilous substance at which this is amphophile.

(2.) *The hyaline cell* (figs. 1, 4, and 17) is the only one of the three elements which has been seen by us to manifest the phenomenon of phagocytosis, that is, which ingests and digests discrete particles. This cell is the "mononuclear" cell of METSCHNIKOFF.* Since, however, the other cells of the Frog are strictly mononuclear, we have thought it wise to adopt a different term. In the resting condition the cell is spherical. The cell-substance betrays no differentiation into ectosarcal and endosarcal portions. Therefore, when this cell becomes active, it may exhibit the wildest irregularities of form. There is no special pseudopodial character, the processes may be simple, or branched, they may be extraordinarily long and attenuated, or mere rounded eminences. Lastly, the cell may become excessively flattened until it is reduced to a protoplasmic film. The cell-substance is very clear and transparent, and free from specific granules. The nucleus is exceedingly characteristic. It presents, when stained, the appearance of a spherical bladder, formed by a very delicate staining membrane, and enclosing a sharply defined spherical nucleolus, which is placed at the centre of the sphere (fig. 4). Fine filaments may often be traced from the nucleolus towards the nuclear capsule, but they are seen with difficulty. When proliferation of these cells occurs nuclei may be found containing two nucleoli (fig. 17).

* 'Leçons sur la Pathologie comparée de l'Inflammation,' Huitième Leçon, p. 131.

The most prominent functional characteristic of the hyaline cell is its power of ingesting and digesting solid particles. At the same time this power is strictly limited, for the hyaline cell is absolutely incapable of ingesting, or even of effecting contact with, for instance, an intact and virulent anthrax bacillus. The bacillus must first be killed or maimed. In the presence of such substances as coagulated proteid, or dead bacilli, the cell exhibits the following well-marked phenomena. The food particle is ingested by pseudopodial activity in the ordinary way, and at first lies merely embedded in the cell-substance. A digestive vacuole is formed round the particle, which floats freely in the vacuolar fluid. The particle, if soluble (*e.g.*, the bacillus or proteid mass) is dissolved, and, lastly, the now empty vacuole closes. In other words, this cell accurately reproduces the ingestive and digestive phenomena of the carnivorous Protozoa.

Though we have not endeavoured to witness the fission of these cells, yet we have found them increase in number in a hanging drop. We have also seen a hyaline cell multiply in a hanging drop by a process of budding, rather than by binary fission. In this case the daughter-cell is about one-third the diameter of the mother-cell. Such young hyaline cells may be seen in hanging drops when leuco-cytosis, that is to say, an increase in the number of cells, has been induced by the presence, for instance, of some microbe (fig. 7).

The hyaline cell is much more easily killed by change of medium than the other two kinds of cells. It, however, exhibits considerable differences in this respect in different Frogs. If the cells be asphyxiated, as, for instance, by putting a cover-slip down on to a drop of lymph, they frequently burst in a way precisely similar to the bursting of the "explosive" or hyaline cells of *Astacus*.* Rarely a similar fate befalls some of the hyaline cells in a hanging drop, and it is probably due to the formation of a very dense clot. In the relative instability of its cell substance the hyaline cell recalls the similar ingestive cell of *Astacus*. In *Astacus*, however, the cell is so unstable that it has been called the "explosive cell." The hyaline cell of *Astacus* is similarly the phagocyte of that animal.

(3.) *Rose-reacting Basophile Cell* (figs. 4 and 5).—This cell, like the similar cell in *Astacus*, is characterized by its relative immobility. We have never seen any signs of active pseudopodial movement. In normal lymph the rose-reacting cells are very few in number (about two per cent. of the total number of cells), and those present are also small as compared with the eosinophile and hyaline cells. As a result of the presence of foreign matter in solution in the plasma, these cells increase both in number and in size, and become highly charged with granules. A similar increase is frequently associated with an œdematous condition of the animal. A comparison between figs. 5A and 5B will render clear the great difference between what may perhaps be called the resting and active condition of the rose-staining cells. The transition from the resting to the active condition will take place in hanging drops.

* HARDY, 'Journal of Physiology,' vol. 13.

In the resting condition the cells are small, spherical, and have a large spherical nucleus. The cell-substance is very scanty, but is charged with granules which are much smaller and very much less refractive than the eosinophile granule. It was in these cells that EHRLICH first described the existence of the fine basophile granule, or δ-granulation.* In the latter character, namely, the duller appearance in the living cell, they resemble the similar rose-reacting granules of *Astacus*. The spherical nucleus either stains uniformly, showing no chromatin filaments, or it encloses a central irregular chromatin mass, from which filaments stretch to the nuclear capsule.

The enlarged active cell is usually angular in shape, and the nucleus is then mostly oval. The cell-substance is highly charged with granules, which, as in the similar cell of *Astacus*, extend quite to the margin of the cell. There is never any trace of an ectosarc. Not infrequently the cell body contains a vacuole. We have never witnessed the manifestation of any kind of ingestive activity on the part of the rose-reacting cell, and the increase in size and number appears to be related solely to the presence of substances in solution in the lymph. Little is known of the distribution of these cells in the body.

In *Astacus*, as has been previously shown,† similar cells inhabit a peculiar tissue, which forms the adventitia of the anterior sternal artery.

In the Frog they are normally present in the connective tissues, and in the Mammalia, probably, identical cells are found in the peculiar adventitia of the arteries of the spleen and grouped about the capillaries, especially of the splanchnic area. Dr. SHERRINGTON exhibited specimens to the Physiological Society, in May, 1891, which showed very similar cells grouped about the blood-vessels of the intestine in cases of cholera.‡

Giant Cells (figs. 11 and 16).—These do not normally occur in the Frog, but are formed at a certain stage after the introduction of microbes into the lymph. Their formation may be followed in the hanging drop, and they are then seen to be very remarkable bodies, produced by the partial fusion of eosinophile cells or hyaline cells, or both. They are, therefore, of a plasmodial nature. As will be seen later, their formation recalls, in its mode and effects, the temporary conjugation of some Protozoa, and similarly these plasmodia ultimately disintegrate into their constituent cells (see Section V.). These plasmodia are produced either by the fusion of (1) eosinophile cells—in this case the fusion is limited to the ectosarc—or (2) hyaline cells, when the fusion of cell-substance is, so far as can be seen, complete.

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* "Beiträge z. Kenntniss d. Granulirten Zellen, &c." 'Verhand. d. physiol. Gesellsch. zu Berlin.' 1878–79, No. 8.

† HARDY, 'Journal of Physiology,' vol. 13, p. 177.

‡ Since writing the above we have found that the basophile cell found in the peritoneal cavity of the Frog differs from the basophile cells found elsewhere in the body. The differences are of two kinds : (1) the peritoneal cells are larger, and (2) the basophile granules which they contain are larger and are well-defined spheres. The peritoneal cells thus show granules belonging to EHRLICH's group γ.

Lastly, there is also (3) a plasmodial mass, formed of both eosinophile and hyaline cells. With regard to the third case we are not prepared to say whether the fusion is complete, a single plasmodium resulting, or whether the plasmodium is double, the eosinophile cells being in contact with an inner hyaline plasmodium.

Various observers have noted the fact that the eosinophile cells are relatively more numerous in Frogs during winter. Our observations have been confined to the summer months, and we have found that the relative number of the different classes of cells varies in different parts of the body. In the lymph from the subcutaneous lymph spaces the eosinophile variety form from 17 to 25 or 30 per cent. of the total number of cells. The basophile cells form about 2 per cent. In the peritoneal fluid the percentage of eosinophile cells is higher, ranging from 30 to 50 per cent.

The histological structure of the sporadic mesoblast of the Frog, excluding the red corpuscles and platelets, which stand on a different footing from the rest, may be summarized as follows :—

Normal	I. Cells normally free in the blood and in the lymph.	(a.) Eosinophile cells, nucleus horse-shoe shaped or lobed; do not ingest particles; but are motile unicellular glands.
		(b.) Hyaline cells, free from specific granulation: nucleus round with central nucleolus. Phagocytic, i.e., they possess the power of ingesting and digesting discrete particles.
	II. Cells are few in number and small in normal lymph. Normally present in the lacunar spaces of areolar tissue.	(c.) Basophile cells, spherical, with scanty protoplasm when small, angular, rounded or flattened when large, cell-substance charged with tiny basophile granules, which give a vivid rose colour with methylene - blue. Large oval or round vesicular nucleus, sometimes containing irregular chromatin mass and filaments.
Abnormal	III. Large amœboid cells, vacuolate, with ingesta frequently in the vacuoles, multi-nuclear, very active and phagocytic.	Giant cells formed by fusion of hyaline cells similar to the large phagocytic cell of *Astacus*.
	IV. Small bodies either round and quiescent or amœboid.	Nucleated cells budded off from the eosinophile or hyaline cells.

SECTION III.

Leucocytosis.

The most constant phenomenon of inflammation in a vascular part is the appearance of an increased number of wandering cells in the tissue outside the blood-vessels. Some of these cells appear on the scene by means of migration from the interior of the blood-vessels, others find their way thither by migration from neighbouring tissues along lymph paths, but the increase is also largely due to the direct multiplication of the cells by fission on the spot. Whether these cells are attracted by means of " chemotaxis " or not, does not concern us here, and we shall abstain

from offering an explanation of the process or cause leading to this collection or infiltration of cells. A question which was of much greater importance to us is the nature of the cells, and the sequence in which these cells appear. On injecting substances like anthrax culture under the skin of the Frog, do the three kinds of cells appear simultaneously, or does one class of cell appear before another?

This is a point which, so far, has been neglected by those who have made "chemotaxis" a subject of special investigation. Being satisfied, or assuming, that the attracted leucocytes are phagocytic in property, they have considered it sufficient to prove that, in certain animals, by means of certain substances, or bacilli, leucocytes are attracted, and positive chemotaxis was considered to be of special use to phagocytosis, because it was tacitly understood that the leucocytes attracted to the spot were always active phagocytes. We shall now show that this conception is true in part only, and that the usefulness of the wandering cells in the conflict against injurious substances does not lie wholly in their phagocytic powers.

We have seen that the Frog possesses three kinds of wandering cells. Of these, the eosinophile cells are never phagocytic, the hyaline cells, on the other hand, are so. If, therefore, the usefulness of "positive chemotaxis" in the battle against micro-organisms lies solely in the fact that thereby phagocytes are attracted, the hyaline cells should be the ones to appear on the battle-field. We found, however, that the cells which first collect in greatest number (or are attracted) are the eosinophile ones, and that it is not until some time has elapsed that the hyaline cells become evident.

On injecting a fresh culture of anthrax bacilli into the subcutaneous tissue of a pithed Frog, and keeping it at the ordinary temperature, which was 12° C. at the time these experiments were performed, and removing drops of lymph with a capillary pipette at intervals of half an hour, it was noticed that the leucocytes which first appeared were the eosinophile ones. They rapidly increased in number until the third or fourth hour after inoculation, and collected in masses around the bacilli. From the third or fourth hour onwards hyaline cells become conspicuous, increasing gradually in number. The eosinophile cells still further increased, and after eighteen or twenty-four hours their number was very great, and often masses of bacilli could be seen surrounded by those cells, and wherever the number of eosinophile cells was greatest, there also the hyaline cells were most conspicuous, and phagocytosis most marked.

The first phenomenon noticed, therefore, after an inoculation with anthrax, is the appearance of eosinophile cells, or, *sit venia verbo*, an eosinophile leucocytosis. Many of these cells certainly find their way to the field of action by migrating from the vessels, others from the neighbourhood, eosinophile cells being always found free, *i.e.*, outside the vessels, in the lymph spaces. Undoubtedly also the cells multiply *in situ*. For, as we shall show later, a multiplication of the eosinophile cells may be easily demonstrated on the slide in a hanging drop; again, all the above phenomena may

be watched in the amputated leg of a Frog, and also if after stripping the skin of a Frog's thigh from the muscles, and tying it at both ends so as to convert it into a closed tube, we inoculate this tube with anthrax bacilli, and keep it at the ordinary temperature. "Chemotaxis," therefore, cannot by itself explain this leucocytosis, for besides an attraction, if such exist, we also have an active proliferation of eosinophile cells.

On repeating the above experiments with cultures of *Bacillus pyocyaneus*, or with beer-yeast, or even with inorganic irritants, such as nitrate of silver, the same result was always obtained : the cells to appear first were invariably the eosinophile leucocytes, and it was only later that the phagocytes increased at the seat of lesion.

Other methods also were employed to show that the eosinophile cells are the first attracted. On placing small sponges dipped in cultures of anthrax, pycyaneus, or yeast, or even in a very dilute solution of nitrate of silver, either under the skin or into the peritoneal cavity of a Frog, after two to four hours eosinophile cells were almost exclusively found in the meshes of the sponge. Lastly, on placing capillary tubes filled with the same substances under the skin, or into the peritoneal cavity of a Frog, after two to four hours the same result was obtained.

It is only after the eosinophile leucocytosis is established that the hyaline cells begin to appear in appreciable quantity, and they now rapidly increase in number. At the same time, however, the eosinophile cells do not cease to increase, so that there is both an eosinophile and a hyaline leucocytosis. The hyaline cells at once become active, and begin to take up the bacilli, and, as we said above, are most active where the eosinophile cells are most numerous. This has been observed so consistently, that even in the absence of better evidence, a correlation between phagocytosis and the eosinophile leucocytosis suggests itself.

The hyaline cells now rapidly increase in number, and after eighteen to twenty-four hours are often very numerous ; they may eventually outnumber the eosinophile cells, and they mostly contain bacillary remains in their interior.

When phagocytosis is at its height we notice yet another change. The rose-reacting cells, which under ordinary conditions are but sparse, are now greatly increased, so that at one time (about the eighteenth to twenty-fourth hour) all the three cellular elements are numerous.

We have then the following changes after inoculating a Frog with anthrax. At first, the eosinophile cells appear and collect around the bacilli (eosinophile leucocytosis) ; then the hyaline cells appear (hyaline leucocytosis) and soon show a keen phagocytic action, the eosinophile cells increasing, however, at the same time ; and latest of all the rose-reacting cells also increase (rose-reacting leucocytosis). We shall attempt to show later the specific value of each of these groups of cells. One form of leucocytosis merges into and overlaps the other, and it is difficult to separate them from each other by any time limit. This much, however, is certain, that we have

never observed phagocytosis with virulent anthrax without previous eosinophile leucocytosis.

If, for some reason or other, the eosinophile cells do not appear, the hyaline cells are apparently powerless. Thus, on warming a Frog which has been inoculated with anthrax, the eosinophile leucocytosis is absent, and though phagocytes appear at times in large numbers, the bacilli will thrive, being left unharmed. It cannot be objected that heat " paralyses " the phagocytes, because some of them did contain bacilli, and they will not refuse Indian ink or vermilion particles, though exposed to a temperature of from 25°–30° C.

Again, on narcotizing a Frog with a mixture of chloroform and ether, and inoculating it, while under the influence of the anæsthetic, with anthrax baccilli, the latter, as KLEIN and COWELL have shown, will often grow well. In some cases, however, even under these conditions, they will refuse to grow. In the latter cases, the eosinophile leucocytosis is always extremely well marked, in the former, the number of eosinophile cells is very small. Here also it cannot be claimed that the chloroform-ether mixture paralysed the phagocytes, because the latter, at times, were present in fairly large numbers, and, though they left the bacilli untouched, they invariably took up spores.

The same was observed when the circulating blood was replaced by saline solution (·75 per cent.). A cannula was tied in the conus arteriosus and the vena cava ant., and the NaCl solution was allowed to circulate under a low pressure for 2–4 hours, and then the Frog was inoculated in its subcutaneous tissue. The bacilli grew in most cases, and then the absence of an eosinophile leucocytosis was marked, although the phagocytes were often present in large numbers, containing, in many instances, spores.[*]

No doubt here, chloroform and ether and the artificial circulation cause changes in the chemistry of the tissues, but it must always remain a significant fact that in the absence of eosinophile cells the bacilli thrived excellently. The result of warming a Frog for a long time, keeping it for instance at 25° C. for a week or more, is quite different from that of a brief warming. If, at the end of the longer period, the animal be inoculated with anthrax, it is found to be immune as in the normal condition, and now, unlike the case of the brief warming, an abundant eosinophile leucocytosis takes place. Lastly, it may be mentioned here, that in many instances a cluster of bacilli was seen to be closely surrounded by a mass of eosinophile cells, with hardly a phagocyte near. The bacilli were then extremely degenerate and broken up, or forming spores, staining very badly or indifferently with methylene-blue.

All these observations tend to show the great importance of the eosinophile cells in the conflict against the bacilli or micro-organisms.

Leucocytosis in a Hanging Drop.—The three stages of leucocytosis can also be demonstrated outside the body on the slide, by means of a hanging drop. On

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[*] Compare footnote to page 296.

removing a drop of lymph from a Frog and inoculating it with anthrax, and selecting a suitable spot for examination, it is seen that the eosinophile cells collect in numbers around the cluster of bacilli. In a successful specimen there is a well-marked increase of these cells, and it is possible at times to watch the division of these leucocytes.

Later on, the hyaline cells approach the cluster, so that at this time the mass of bacilli is surrounded by both eosinophile and hyaline cells. The former appear to be present in larger number. On examining the hanging drop 12–18 hours after inoculation, staining it previously with a rapidly fixing solution of methylene-blue, a decided increase in the number of rose-reacting cells is noticed. The latter cells are found in all stages of development, from the small round form to the large and very granular one.

On the slide therefore, as within the body, the phenomena are identical, and in each case the inoculation with bacilli is followed first by a collection or aggregation of eosinophile cells, next the hyaline cells appear in appreciable numbers, and the rose-reacting cells also increase at the scene of action.

We have, in the hanging drop, also seen that whenever the initial eosinophile leucocytosis was absent, the hyaline cells did not exert any activity and were not attracted in numbers. On the other hand, whenever the eosinophile cells had collected around the bacilli in large numbers, in all typical cases the hyaline cells streamed towards the bacilli and ingested them eagerly. Here, therefore, we again find that there exists a distinct correlation between phagocytosis and eosinophile leucocytosis.

The correlation between the increase in number of the basophile cells and the other kinds of leucocytosis is much more difficult to prove, because it seems that on simply keeping a drop of lymph over night in a moist chamber these cells will increase, though not in the same measure as after a previous inoculation with anthrax.

Again, on inoculating a drop of lymph with a solution of albumen, the rose-reacting leucocytosis is well marked, though an increase in the eosinophile cells may not be observed.

The question naturally arises as to how far the three kinds of leucocytosis are really distinct, as to how far they may occur independently.

There appears to be little doubt that an increase in the numbers of one or other of the three types of cells may occur in the absence of a corresponding increase in the numbers of the remaining kinds.

In œdematous Frogs the lymph is usually highly charged with rose-reacting cells, and this may occur with a diminution in the number and vitality of the eosinophile and hyaline cells. Similarly the injection into a lymph sac of finely divided and sterile coagulated proteid, such as is produced by boiling a solution of egg albumen, leads to an enormous increase in the number of hyaline cells, without a corresponding increase in the eosinophile cells. On the other hand, we have never witnessed an

eosinophile leucocytosis unaccompanied by a subsequent increase in the numbers of the other cells.

Phagocytosis.

Having thus far described the general changes which follow on an inoculation with bacilli, and the order in which the various cellular elements appear at the seat of injection, it remains now to discuss the minute changes and the parts which the different cells play in the conflict. Before doing so we must indicate what we consider to be the proper phenomena connoted by the term "phagocytosis."

M. GREENWOOD'S study of the Protozoa, and particularly of Amœba, has made it possible to give to this word a precise meaning.* The phenomena which follow the ingestion of a particle by one of the Protozoa are as follows :— (1) If the particle is digestible it at first lies embedded in the cell-substance, then a vacuole is formed about it so that the food mass now floats freely in a digestive fluid. Solution of the particle is more or less completely effected, and lastly, the vacuole closes, and if there be any insoluble remnant it is extruded. (2) If, however, the ingested body is insoluble the physiological reaction is, as it were, incomplete, and, as in the stomach of the highest animals in similar circumstances, there is no secretion of a digestive fluid, and therefore no formation of a vacuole.

Thus the salient feature of the process is the inclusion within the cell's body of discrete particles. If the ingesta be of a nutrient nature then certain well-defined phenomena follow, namely, the formation of the digestive cavity and the digestion of the fragment. If, on the contrary, the ingesta are insoluble, then the phenomena stop short at the ingestive act. Following these lines we would define phagocytosis as being primarily the inclusion of discrete particles in the body of a cell. This may be followed by the formation of a digestive vacuole and the solution of the particles This is the complete process of phagocytosis ; or the included particles may simply remain embedded in the cell's body, and to this incomplete act the term must also be applied. In brief, phagocytosis implies an intra-cellular process. Extra-cellular digestion may occur, but it is not phagocytosis.

SECTION IV.

(1.) We shall now proceed to describe the appearances and changes in a hanging drop of Frog's lymph, kept on a moist stage without being previously inoculated.

On preparing a hanging drop as described above, and examining it under a high power (ZEISS D or E, oc. 4) we see that it is rich in cellular elements. These consist of the wandering cells with which we are dealing, together with some red blood corpuscles. The eosinophile cells at first sight usually appear to be most numerous, owing to the striking appearance presented by their refractive granules. the rose-

* 'Journal of Physiology,' vols. 7 and 8.

reacting cells are by far the least numerous. It is, however, by no means easy to recognize the latter in the fresh unstained condition ; but we have, by means of stained control-specimens, convinced ourselves of the great scarcity of these cells at this time.*

It may here be remarked that good and healthy lymph clots rapidly, and that this property may serve as a criterion of the excellence of the lymph. It was often found that when our Frogs were diseased their lymph refused to clot, and, on the other hand, whenever lymph refused to clot, the changes and phenomena to be described later did not appear.

The drops were placed under the microscope as speedily as possible. It was then noticed that the eosinophile cells at once became active, throwing out pseudopodia. Their movements are rather sluggish, and the change of form is also a slow one. The cell will soon again retract its pseudopodia and become spherical, and then once more throw out pseudopodia. It will thus alternately change its shape, and cease to do so only when it is apparently dead, being then spheroidal and regular in outline (fig. 3).

The hyaline cells are more numerous, but are less readily seen. As a rule they are very active, and are seen to wander about the field and to throw out long and thin pseudopodia.

It is, as we have just said, extremely difficult to recognize the rose-reacting cells in unstained drops, the regular spherical nucleus surrounded by a granular protoplasm being the only guide. In an ordinary drop they are apparently quite inert. They, however, increase in number and size.

Effect of Inoculating a Hanging Drop with various Substances.

By noting the different effects produced by different substances, when added to a hanging drop of Frog's lymph, one is soon convinced that these substances might be divided into classes according to whether they affected more particularly one or other of the different cells. In one class would be grouped those substances which are completely unnoticed by the eosinophile cell, while they were readily ingested by the hyaline cell. Indian ink, anthrax spores, and coagulated proteid are instances. Another class would include those bodies which attract both kinds of cells, such as vermilion, which has a slight action on the eosinophile cells, and is readily incepted by the hyaline cells, and yeast cells, which have a very pronounced attraction for the eosinophile cells, and are taken up by the hyaline cells to a relatively limited extent. Yet another class would embrace substances which at first profoundly attract the eosinophile cells, and only after they have been subject to their influence can become the prey of the hyaline cells, such as active growths of *B. anthracis* and *B. fila-mentosus*. Lastly, there would be the soluble substances, the action of some of which, at any rate, is, like that of egg albumen, mainly limited to the rose-reacting cells. But

* Compare the relative numbers of the different cells given at the close of Section II.

the completion of such a series can only be contemporaneous with the attainment of a full knowledge of the functions of the various wandering cells, and, for the present, we can only note the differential action of various substances when introduced into the body, or into a hanging drop of lymph.

The effect of some of the substances, which we have so far examined, may now be given in detail.

(a.) *Inoculation with Anthrax Spores.*—When a drop of lymph is inoculated with anthrax spores obtained from an old agar-agar culture, many of the spores are at once taken up by the hyaline cells present.* A single cell may take up several of them. The spores are apparently destroyed by the phagocyte, for, on allowing the specimen to rest at the temperature of the room (15–20° C.), while many of the spores developed into bacilli—a process which can easily be watched under the microscope—these spores were invariably extra-cellular ; in no case did we observe spores, which had been ingested, develop into bacilli. Food vacuoles were, in many cases, observed within the cell around the spore, showing that these bodies were being digested. The hyaline cells seemed to take up the spores immediately without the intervention of the eosinophile cells, there being thus, as we shall show, a great difference between the ingestion of bacilli and the ingestion of spores. It is difficult to say whether or no the eosinophile cells exert any influence on the spores, but this much is certain, that the spores may be taken up and digested by the unaided hyaline cells. Nor did we notice a movement of the eosinophile cells towards a mass of spores collected on the cover-glass, like that which occurs in the case of the bacilli.

On the other hand, when the spores had grown out into bacilli, the eosinophile cells were at once seen to move towards them and attack them in the manner we shall describe later when we come to discuss the conflict between the cells and the bacilli.

(b.) *Inoculation with Indian Ink.*—The particles are completely unnoticed by the eosinophile cells, but are readily ingested by the hyaline cells.

(c.) *Effect of Finely-divided Coagulated Proteid.*—The results are most interesting, and may be mentioned here, though they are most useful as aiding us to interpret the phenomena of repair.

The coagulated proteid was prepared by dissolving white of egg in tap water, filtering, and then boiling the filtrate. An exceedingly fine precipitate may be thus produced. If this be injected into a lymph sac of a Frog, or added to a hanging drop, it is found to induce an excessive leucocytosis, which is almost limited to the hyaline cells. These ingest the exceedingly fine proteid particles, and mass them into balls which superficially resemble a specific granulation, and stain blue with methylene-

* The observations of WARD, on the destructive action of light on anthrax spores, which have been published since the MS. of this paper was completed ('Roy. Soc. Proc.,' vol. 54, p. 472) make it possible that the spores, which were so readily ingested by the hyaline cells, were already dead. It will be noticed that the spores used by us were obtained from old dry (agar-agar) cultures.

blue. For the present, the important point to notice is that the injection causes a proliferation, or leucocytosis of the hyaline (ingestive) cells.

(*d.*) *Effect of Solution of Egg Albumen.*—This has been only partially investigated, and one result will alone be emphasized here. If a sterile solution of egg albumen be injected into a Frog, or added to a hanging drop of Frog's lymph, it results in an abundant leucocytosis characterized by an increase in the number and size of the rose-reacting cells.

(*e.*) *Inoculation with Vermilion.*—In the hanging drop we have observed that the phagocytes may take up vermilion particles without the intervention of the eosinophile cells. But we never saw a destruction or digestion of the pigment particles, so that it is quite possible that the hyaline cells simply take up vermilion granules in order to carry them away without destroying them. We can, however, safely conclude from our experiments that the vermilion particles may be taken up by the hyaline cells without the intervention of the eosinophile cells.

These observations were controlled by experiments in the body. Anthrax spores and vermilion particles are rapidly taken up and carried away, without a previous eosinophile leucocytosis at the seat of inoculation. It is often stated that the spores first develop into bacilli and are then taken up by phagocytes. Observations on the living (pithed) Frog, however, show that most of the spores are taken up by cells, and at once destroyed. Many of the extra-cellular spores, however, develop into bacilli and are subsequently destroyed.

Saline solution injected subcutaneously does not lead to any apparent changes, the eosinophile cells are not increased, and the hyaline cells seem to be unaffected.

Section V.

The Behaviour of the Cells towards Active Growths of Bacillus anthracis *and* B. filamentosus.

We shall now proceed to describe, in detail, the phenomena observed in a hanging drop following on an inoculation of bacilli (and their products). *Bacillus anthracis* and *Bacillus filamentosus** in fresh cultures, readily grow into long chains, which form very convenient objects for continued observations. These bacilli are non-pathogenic, so far as the Frog is concerned, but they by no means react as indifferent substances, since when injected under the skin they always lead to a typical inflammation.

It will be seen that the phenomena observed when these bacilli are used are widely different from those noticed when innocuous substances are employed. With very slight differences the appearances are the same, whether anthrax or filamentosus bacilli are employed.

* This bacillus has been separated by KLEIN, who kindly gave us a culture. It grows in beautiful filaments, being, however, non-pathogenic.

On inoculating a drop with a trace of bouillon culture (the latter should be fresh, preferably not older than twenty-four hours), and observing a suitable chain of bacilli, we find the eosinophile cells which happen to be in the neighbourhood travel towards the chain, performing slow amoeboid movements during their progress. Some of the cells apparently come from some distance. This may be an attractive influence exercised on the cells by the bacilli and their products, or it may be the expression merely of the active movements induced by the change of medium. When the cells reach the chain or individual bacilli, they at once become extremely active, extending and retracting their pseudopodia, and a constant streaming of the granules may often be observed. The granules, moreover, may be discharged, or at least wholly disappear, so that the same cell at one time appears highly granular, at another almost hyaline. Then suddenly the cell will apply itself along the bacillus and, so to speak, pour itself along it, being in some cases applied to one side of the rod or chain, in others surrounding it, and yet in others fixing itself at the extremity of a bacillus. (Figs. 10, 13, 14, 15, 17.)

At first sight it seems as though these cells had taken up the bacilli, but careful or continued examination will easily prove that this is simply an appearance. The bacilli can, in most cases, be clearly seen to lie on or under the cell, or the cell is, so to speak, folded around the bacillus, and, if from any cause the cell contracts to a sphere, the sphere does not enclose the bacillus but only touches it. Moreover, as we shall show later, the eosinophile cell eventually leaves the bacillus, handing it over to the hyaline cells to be devoured. In fact, almost numberless observations have proved to us the correctness of METSCHNIKOFF's* statement that the eosinophile cells are never phagocytic. We have tempted them with charcoal, vermilion, Indian ink, with many kinds of bacilli and cocci, with curare and other substances, but they refuse all things alike.

Thus the eosinophile cell comes into apposition with the bacillus and streams along it, losing its previous spherical shape. It is now more or less fusiform, so that we have the appearance shown in figs. 10B and 17. In many cases by focussing one can detect the bacillus and determine with ease that it does not lie *in* the substance of the cell, but in other cases it is impossible to recognize any trace of the bacillus in the area occupied by the cell.

The latter will remain motionless for some time, the only change noticed being a continual fluctuation in the appearance and number of the granules. After a time the cell may again change its shape, becoming once more spherical, soon to assume another shape, and once more to "pour itself out" along the chain.

During this time other cells apply themselves along the chain, passing through the same phases. Others, on the other hand, simply swarm around the chain, so that at one stage the latter is both actively attacked by some eosinophile cells and closely

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* 'Leçons sur l'Inflammation.' Paris, 1892, p. 136.

surrounded by others. In the case of the later arrivals the loss of granules is not so complete. This may be said to be an inverse function of the number of cells attacking the chain. The mass of eosinophile cells gradually contracts, so that the result is a "plasmodial" mass hiding the chain (figs. 11, 11A, &c.). It should be stated, however, that some of the eosinophile cells may leave the bacilli soon after they have poured themselves along the chain.

The next phase is the approach of the hyaline cells or phagocytes. Several of these come up and become hidden in the plasmodial mass, which now presents the appearance of an opaque rounded or nodulated mass with separate heaps of granules on the surface (fig. 11B). The contact of a fresh cell, as it comes to lose itself in the mass, acts as a stimulus, and causes active streaming and writhing movements. These become fainter and fainter until they are re-awakened in their first intensity by the arrival of another cell. Now gradually one eosinophile cell after another separates from the mass and moves off. Eventually we find that all which is left behind is a plasmodium of the hyaline cells containing fragments of bacilli enclosed in vacuoles, thus showing that an actual digestion is going on (fig. 16B). The chain of bacilli has thus succumbed. The digestion of the bacillary fragments is completed, and finally, at any rate in some cases, the plasmodium of hyaline cells breaks up into separate cells.

This is the description of a typical conflict. We have thus two stages—(1) the attack by the eosinophile cells; (2) the ingestion and digestion by the hyaline cells.

The attack by the eosinophile cells is an active one, and one which must be carefully distinguished from phagocytosis. Phagocytosis it is not, because we have neither ingestion nor digestion. There can be no doubt that the eosinophile cells have a distinctly harmful action on the vitality and growth of the bacilli, for it is seen that in cases where a chain is attacked by a sufficient number of these cells, without the subsequent access of hyaline cells, all growth is suspended. The bacillus apparently dies, or, at any rate, nutritive activity is impaired, because, ceasing to grow, it will either form spores or become absolutely unstainable. An actual solution or destruction of the bacillus by the eosinophile cells has not been observed, but a chain will often be seen to be broken up into fragments, or to become diminished in size.

In many specimens, where the number of eosinophile cells was large and the hyaline cells sparse, no growth was noticed anywhere in the drop, but many plasmodial masses were observed, within which the bacilli were hidden.

The eosinophile cells proliferate, cell division being not infrequently observed, and young stages being by no means uncommon. They also seem to possess a high degree of vitality, for eosinophile cells which apparently had done their duty were seen to leave the plasmodial masses or the chain of bacilli, and to direct themselves to another field of action.

At the end of the process the number of rose-reacting cells is greatly increased, cells in all stages often being found in great numbers.

We have, by numerous observations, convinced ourselves that these phenomena are not simply stage phenomena, but that they also occur in the organism. By removing lymph at different intervals from the seat of inoculation of a Frog injected with anthrax, all the various stages can be easily obtained.

These experiments then throw new light on phagocytosis, showing that previous to the ingestion and digestion of the bacilli there is an attack by the eosinophile cells, which apparently prepares the bacilli to be taken up by the hyaline cells. That this is actually the case, observations in cases where the bacillus is victorious will show.

In most hanging drops there are always some parts where the bacilli are not destroyed or impaired, but develop typically. Now here there has been either a total absence of an attack by the eosinophile cells, or a very incomplete one, the cells, after a feeble attempt, leaving the bacillus to its own fate. A part of a chain actually in contact with or surrounded by an eosinophile cell, however, seems to be hopelessly beaten and refuses to grow, though rapid growth may occur in the unattacked parts of the same chain. Lastly, we have never seen a hyaline cell take up and digest a bacillus or chain of bacilli that had not previously been attacked by the eosinophile cells.*

The following is a detailed description of a few among our many observations :—

Experiment I.

DROP I.

Two drop cultures made from lymph from thigh of a Frog.

Hanging drop inoculated with a small quantity of anthrax. A moist chamber was made, as described in Section I., and over this was placed a sterilized cover-slip, with a tiny drop of bouillon from an anthrax culture on the under surface. Lymph was then taken from subcutaneous spaces of the thigh with a fine pipette, a drop rapidly added to the anthrax, and the cover-slip again inverted over the chamber. The preparation was then as quickly as possible brought on to the stage of a microscope and examined with ZEISS ob. D, oc. 4. From time to time sketches were made (cf. figs. 11, 11A, and 11C).

Watched a long chain of bacilli in the centre of the field. Two eosinophile cells came to each end, and one to the middle of the chain. Five cells in all.

As soon as the cells reach the bacillus rapid streaming movements occur, and spread the cell-substance along the chain. Rapid discharge of granules.

Half hour.—Eosinophile cell at one end divided. Daughter-cell free from granules. It moved away from the chain. Within the next half-hour the same cell was seen to divide again. Also one of the cells at the other end of the chain was seen to divide.

Two more eosinophile cells have reached the chain, on which there are now seven cells.

Chain broke in two. Two eosinophile cells are on the north piece, and this was watched.

1 hour.—A hyaline cell has now moved to the chain and ingested one end. It has pushed out a long pseudopodium to the other end of the chain. (The chain is slightly bent, fig. 11.)

* This is only true when freshly made cultures of virulent bacilli are used. Compare Section IV., p. 295, and Section VI., on the conflict with yeast torulæ.

The pseudopodium is being contracted, thus slowly bending the chain.

The hyaline cell is displacing the eosinophile cells and more completely ingesting the chain; part of the chain still attacked only by eosinophile cells.

A third eosinophile cell rapidly moves towards the mass, and then throws out a delicate fringe of pseudopodia, which touch the mass, finally fusing with it. The contact of the new cell provokes violent streaming movements. Another eosinophile cell fuses with the mass (fig. 11B).

The mass has now become rounded, the eosinophile granules have been re-formed, and we have four heaps of granules representing the four eosinophile cells on the surface of the mass. The hyaline cell and bacillus are completely hidden.

N.B.—The last two eosinophile cells, and all those which join the mass later, do not completely discharge their granules.

2 hours.—The whole field of the microscope has now become very full of cells, all of which, both eosinophile and hyaline, are in active amœboid movement.

Churning streaming movements of the mass slacken. A fifth, and then almost immediately a sixth eosinophile cell fuse with the mass, their onset, as before, causing violent streaming movements. Blunt pseudopodia are also thrust out.

Sometimes the mass becomes lobed, and one sees a heap of very numerous, large, and very refractive granules in each lobe. Again all outlines fade, and the spherical mass has a remarkable refractive, curdled appearance, and is very opaque (fig. 11c).

2 hours 20 minutes.—A second hyaline cell has fused with the mass.

2¼ hours.—Preparation accidentally shifted. Low power put on to find the mass again. This is quite impossible, for the whole drop is now studded with similar masses. There are also a great number of free eosinophile cells. An enormous multiplication of these cells has taken place. They are all heavily laden with granules. No free bacilli.

3 hours.—Another mass chosen for watching; a large one (fig. 11D). Hyaline cells very extended and numerous.

3¼ hours.—Hyaline cells exceedingly numerous and exceedingly irregular. Some which have wandered into the field are very large, and show vacuoles which contain fragments of bacilli. They are *very* active (fig. 12).

The mass all this while assuming more and more the appearance of a heap of cells. All pseudopodial and streaming movements ceased.

The mass now looks like a heap of eosinophile cells. These are separating from one another and are moving away.

4 hours.—Twenty-five minutes later the eosinophile cells are gone, and disclose a large central hyaline cell. This stage carefully drawn with camera lucida (fig. 11E).

4½ hours.—Drop examined, and shows general phagocytosis. Observation discontinued.

<center>DROP II.</center>

Contained, in addition to scattered chains, one large mass of anthrax filaments.

Cells very much less abundant than in Drop I.

Three eosinophile cells attacked a cluster of anthrax filaments. Owing to relative paucity of cells the greater part of the anthrax in the field remained unattacked.

One cell watched attacked a mass of bacilli, and streamed along a chain showing active movements.

Movements gradually ceased, and the cell contracted into a ball, *which did not enclose the bacillus but remained just touching it.*

After a prolonged period of absolute quiescence the cell again became active, and now moved away from the bacilli. It twice changed its line of movement to avoid bacilli, and finally came to rest in an isolated position.

During the third and succeeding hours the anthrax grew with immense rapidity.

The notes of another drop-culture experiment, in which the phenomena were followed to the final dissolution of the plasmodium of hyaline cells and the close of phagocytosis, are as follows :—

Experiment II. (figs 16, 16A and c).

Drop culture made with subcutaneous lymph and observation started at 11.45.

11.45.—Eosinophile cells moved up to a chain.

A hyaline cell is near the south end of the chain.

There are now six eosinophile cells on the chain.

12.30.—The granules are constantly changing. Only four distinct cells now, three having fused.

12.35.—The movements of the eosinophile cells have bent the chain.

12.45.—Fusing of the eosinophile cells only partial. Can now count seven cells on chain.

A phagocyte at the south end passes into the mass and is soon lost sight of, as the eosinophile cells went all round it, moving *en masse* in a churning manner.

1.0.—Another phagocyte came into the field at the north end but wandered off again.

1.15.—The eosinophile cells are turning round and round, so that at one time it appears as though there were three or four cells, and then shortly eight or more.

1.40.—The eosinophile cells are separating. One already gone. A minute later another eosinophile cell went off with a sudden jerk and then came back.

1.45.—Central hyaline cell seen now. A phagocyte in the field has ingested a pigment mass. It is vacuolated and the vacuoles contain fragments of bacilli.

The same eosinophile cell left again and again came back.

2.12.—The eosinophile cells are leaving. The hesitating cell gone at last. Only one eosinophile cell left.

2.17.—Two hyaline cells have come from outside and fused.

2.35.—A fourth hyaline cell has joined.

3.35.—The hyaline cells now form a mass with food vacuoles (16B).

5.45.—The hyaline cells separating. Digestive vacuoles collapsing.

6.25.—Vacuoles collapsed.

As an instance of a case where the bacteria have ultimately triumphed, owing to their being present considerably in excess of the eosinophile cells, we may take the following experiment. In all such cases the conflict may be followed up to a point where the accumulation of bacterial products produces a complete paralysis of the eosinophile cells. Naturally this may occur at any stage in the conflict, and we have witnessed instances where the initial dose, so to speak, has at once paralysed the cells and instances which might aptly be styled drawn battles. In the latter the conflict will endure for hours, the eosinophile cells killing some of the bacteria, while in others the bacteria are so far worsted that they have to resort to spore formation.

Experiment III.

DROP CULTURE MADE CONTAINING A LARGE QUANTITY OF *Bacillus filamentosus*.

11.15.—Scarcely any eosinophile cell with granules. In one case saw the granules shrink in size, leaving vacuoles.

11.30.—One long chain in the field stretches up from the bottom of the drop where cells mostly are into an almost cell-free region.   Three cells on this chain.   When first seen they were quite free from granules.   Now they are re-forming them.

11.35.—Cells are moving up the chain and others are approaching the lower end.

11.38.—The eosinophile cells generally are re-loading themselves with granules.

11.40.—The appearance of the hyaline and eosinophile cells in the preparation is very striking (*cf.* fig. 18).   Upper part of the chain still unattacked.   A fourth cell is streaming up from the lower end.   Another chain in the field, which lies lower down in the drop, is already closely surrounded by 12 granular eosinophile cells.

From this a short branch chain extends upwards which is free from cells.

The cells are all moving very sluggishly.

11.55.—The last cell to come on to the chain (No. 4) is hopelessly defeated.   It bursts up and only droplets are left.

12.45.—No further attack has taken place.   Cell No. 3 looks very bad and curdled.   The bacilli are not growing.   Two cells still on the chain are spherical, as are also the free cells.

1.10.—Some of the 12 cells on the deeper-lying chain are still extended.   Others are rounded.

2.30.—Bacillus growing now where it has been unattacked.   The chain which was attacked by 12 cells appears quite dead, but the short chain projecting upwards has increased in length.

Later.—Very rapid growth of bacilli.   The chain attacked by the 12 cells, however, is completely dead.   Of the other chain part was killed, and part which was exposed for only a short time to the attack of the eosinophile cells grew.

(The growth of the bacilli was always determined by making a plan of the chains and numbering the rods.)

The fact that the bacilli are attacked at first exclusively by the eosinophile cells was clearly shown in the following way :—About 0·2 cub. centim. of a fresh bouillon culture of anthrax was injected into the peritoneal cavity of a pithed Frog.   When 20 minutes had elapsed the cavity was opened and film preparations made, and stained with eosin and methylene-blue.   On counting the cells in these preparations, it was found that no less than 82 per cent. of the eosinophile cells were in contact with bacilli.   On the other hand, only 2·6 per cent. of the hyaline cells were even in contact with bacilli.

In a second experiment of a similar nature it was found that 42 per cent. of the eosinophile cells were in contact with bacilli, and only 2 per cent. of the hyaline cells.

The cells were counted with Oc. 10, Ob. $\frac{1}{12}$ apochromatic oil immersion, by POWELL and LEALAND.

The phenomena seen in a hanging drop of lymph inoculated with anthrax or *Bacillus filamentosus* show clearly that the intruding organism is always attacked first by the eosinophile cells before the hyaline cells attempt to ingest them or their remains.   But we are able to go a step further than this and say that the hyaline cell is incapable of ingesting an anthrax or filamentous bacillus before it has been killed or maimed by the eosinophile cell.

The sequence of events observed in experiments similar to those described in detail would afford strong presumptive evidence for this statement, and we find further support in noticing the different behaviour of the two kinds of cells towards the

bacteria and indifferent particles when they are present together in the hanging drop.

We have already seen that the eosinophile cells are not at all phagocytic, and that they are completely unaffected by the presence of indifferent particles in the plasma. On the contrary, the hyaline cells are attracted by such particles as vermilion, Indian ink, or coagulated proteid. They ingest them, and, if possible, digest them.

Now, in the drop cultures, we find that the phagocytes are never attracted by the fresh bacilli. They are even repelled by them. We have seen a freshly budded phagocyte come into the near neighbourhood of an anthrax chain (fig. 8) and there exhibit active pseudopodial movements, which neither resulted in locomotion of the cell, nor in effecting contact with the bacillus. After a while the movements slowed and ceased. Then they were renewed and now the cell moved away from the bacillus.

Bacilli and Indian Ink or Vermilion.—In a drop of lymph inoculated with bacilli and with Indian ink or vermilion, we see strikingly displayed the differential activity of the two kinds of cells. The phagocytes will readily ingest the indifferent particles, while the eosinophile cells with even greater readiness attack the bacilli.

Bacilli and Spores.—This is only true of the kinetic phase of the life-history of the bacillus. The potential form, the spores, are at once ingested by the phagocytes,[*] and in a hanging drop, inoculated both with bacilli and spores, we witnessed the eosinophile cells attacking the bacilli, while the phagocytes ingested the spores. In illustration of this we may quote the notes of the following experiment :—

Experiment IV.

A drop of Frog's lymph was inoculated with fresh bouillon culture of anthrax, and also with anthrax spores from an old agar culture.

3 P.M.—Spores taken up rapidly by hyaline cells without intervention of eosinophile cells—quickly vacuoles are formed, and some of the spores are being digested. At the same time the usual attack of eosinophile cells on the bacilli took place.

7.45.—Many extra-cellular spores have become bacilli.

9.30.—Eosinophile cells have attacked the newly-developed bacilli and everything is proceeding as before. There appears to have been a proliferation of eosinophile cells.

But to prove the statement that the destruction of anthrax and *Bacillus filamentosus* by Frog's lymph is due primarily to the eosinophile cell, it was necessary in some way to paralyse the eosinophile cell while leaving the phagocyte unharmed, and then show that under such conditions, namely, in the absence of the eosinophile attack, the phagocyte is powerless and the bacteria grow freely in the lymph. We have been enabled to do this in two ways.

Action of Heat.—It has already been shown (Section III.) that when a Frog is warmed to a temperature of 25–35° C. it becomes susceptible to anthrax. We there-

* *Cf.* note, p. 296.

fore tested the effect of heat on the processes taking place in our drop cultures, and found that the eosinophile cells are completely paralysed by a rise of temperature, and became quite incapable of attacking the bacteria. *At the same time the phagocytes retain their activity and freely ingest Indian ink particles or spores present in the same drop.* The thermometer of the warm stage indicated a temperature of 35 to 37°·5 C. in our experiments.

Action of Urari.—Turning to poisons, we obtained a similar differential action with urari. This drug produces a marked alteration in the eosinophile cells: their eosinophile granulation becomes changed into an amphophile granulation. This change occurs both in the body and out of the body with a sufficient dose of the drug. In the body, after a while, recovery takes place, and eosinophile granules are again found. In the same way, owing probably to the elimination of the poison, the neuro-muscular mechanism will, at length, recover, and the animal regains the power of movement. Out of the body in the hanging drop the cells appear incapable of recovery, and the amphophile granulation persists.

If a drop of lymph, taken from the body of a urarised Frog while the amphophile granulation persists, be inoculated with anthrax bacilli, we find that the bacilli are not attacked by the eosinophile cells, and after a time they grow.

Abnormal Lymph and Bacilli.—Lastly, among Frogs which have been long kept in confinement in the laboratory and starved, individuals are sometimes found more or less œdematous, and whose lymph contains few eosinophile cells incompletely charged with granules which are sometimes largely amphophile. *B. filamentosus* has been found to multiply in the body of such animals, and, ultimately, to kill them, and both filamentosus and anthrax bacilli will grow freely in drop cultures of lymph taken from such an animal. At the same time hyaline cells are present, and have not lost their phagocytic power. Take, for instance, the following experiment.

Experiment V.

A drop of non-clotting lymph from a Frog which had been a long time in the laboratory was inoculated with anthrax bacilli and anthrax spores.

The eosinophile cells did not act at all, and therefore the hyaline cells made no attempt whatever to ingest the bacilli.

At the same time the spores were ingested as usual by the hyaline cells.

The bacilli grew rapidly.

Ingested spores were seen to be lying in distinct food vacuoles, and many were undoubtedly digested.

How essential the removal of the dead bacterial remains by ingestive solution on the part of the phagocytes must be in the body, we see when we watch hanging drops made from lymph either abnormally deficient in hyaline cells or in which the hyaline cells are abnormally sensitive to a change of medium. The relative number of the two kinds of cells varies in lymph drawn from different parts of the body. Occasion-ally in hanging drops made with peritoneal lymph hyaline cells may be almost absent.

Under these circumstances the eosinophile attack takes place and the bacilli are killed, but there are no scavengers to clear away the traces of the fray, and the field remains strewn with bacilli, which, if they are not dead, have at any rate completely lost the power of growing in the drop.

The Changes in the Specific Granulation of the Eosinophile Cell.

The Amphophile Granule (fig. 17A).—The facts discovered by following the phenomena exhibited in a hanging drop, were supplemented by the examination of such drops, or of lymph taken from the body, when fixed and stained with the methylene-blue solution, or with eosin and methylene-blue at different intervals after inoculation.* The same phenomena, the attack of the eosinophile cell, the formation of plasmodial masses, and the final phagocytosis, are again seen, but new light is thrown on the glandular nature of the activities of the eosinophile cell. In the attack on a bacillus the cell discharges, more or less completely, its granules. The amount of discharge depends on the strength of the stimulus. If, for instance, a considerable number of cells attack a bacillus, then the discharge will be only partial in many of the cells. The process of the discharge of granules was followed with high powers. If a cell attacking bacilli be watched under Oc. 4, Ob. $\frac{1}{12}$th, the granules will be seen to travel either singly or in groups of two, three, or four, from the central mass of granules to those parts of the cell which are immediately in contact with the bacillus. There they will be gradually seen to shrink in size until they disappear. This phenomenon, namely, the diminution in size and loss of granules, can be demonstrated in preparations fixed with the methylene-blue solution, or in films which have been rapidly fixed by heat, or by corrosive sublimate. As compared with the rate of loss of granules in, for instance, the secreting cells of a salivary gland, the process is very rapid. After the discharge the cell often re-forms its granules. Tracing these events by means of the basic and acid dyes, we find that the re-formed granules are at first amphophile, and only towards the close of the conflict do they become truly eosinophile.

But the amphophile substance is a stage, not only in the construction of an eosinophile granule, but also in its destruction. The action of urari may again be cited, for if this drug be added to lymph, the eosinophile granules alter to amphophile granules, and the change may be followed in a hanging drop of lymph to which has been added a little urari, and a trace of methylene-blue. We have watched the granules in a cell become slightly smaller and less refringent, and then colour with the dye. Similarly, when urari or bouillon containing a considerable quantity of bacterial products is added to the lymph, a change from eosinophile to amphophile granulation rapidly occurs. In the latter case, if the poisons be not present in too great quantity, the eosinophile condition is re-established.

* Preparations stained first with eosin and afterwards with methylene-blue were made according to EHRLICH's film method.

When the conflict with the micro-organisms is carried out swiftly to a successful issue, the changes appear to be as follows :—

i. The initial sluggish eosinophile cell.

ii. The discharge of the granules, either completely, when the cell effects contact with a bacillus, or so far as to make them amphophile.

iii. The reconstruction of amphophile matter by those cells which have completely discharged, the amphophile matter being a stage in the elaboration of the eosinophile granule.

iv. The complete regeneration of the eosinophile granules.   And this occurs in what may be spoken of as the plasmodial stage of the conflict.

The change of the granulation of the eosinophile cell from eosinophile to amphophile and back again to eosinophile may be illustrated by the following experiment.

Experiment VI.

A series of anthrax drop cultures were made with the lymph from the same Frog.   These were stained at different intervals with the methylene-blue solution.

(*a.*) Stained as soon as possible after the addition of the lymph to the drop of bouillon culture. Eosinophile cells amœboid, granules amphophile or discharged.   Many chains already attacked by eosinophile cells.   Hyaline cells amœboid.   Rose-staining cells present (fig. 17).

(*b.*) After fifteen minutes.   Eosinophile cells with eosinophile granules present.

(*c.*) After thirty minutes.   Eosinophile cells present, with perfectly eosinophile granules (*i.e.*, they absolutely refuse to stain).   Where a cell is in contact with a bacillus it contains granules which are irregular in shape, are being discharged, and which stain.

(*d.*) After forty-five minutes.   Where the eosinophile cells are in contact their granules are amphophile (blue-staining), where they are floating freely they contain eosinophile granules.

(*e.*) After one hour.   Some eosinophile cells applied to chains are still quite free from granules. Contents of the bacilli no longer stain as a homogeneous blue substance.   Disintegration has commenced, and the staining is in patches.   Free amphophile cells rare.

(*f.*) After one-and-a-quarter hour.   Rose-staining cells in marked abundance.   Giant eosinophile cells (plasmodia) present, always with heaps of truly eosinophile granules.

The series was continued up to $3\frac{1}{2}$ hours, and the most prominent feature was the increase in the rose-staining cells.

SECTION VI.

Action of the Cells on Yeast Torulæ.

There are present then, in the body of the Frog, two kinds of wandering cells, the glandular eosinophile cell and the ingestive hyaline cell, and the most superficial study of these cells shows that the former is much more stable and can endure much greater changes in its environment than the latter.   The eosinophile cells will persist as spherical bodies, with granules intact, for days in a drop of lymph, and for long after the other elements have disintegrated.   They may or may not be dead, but

absence of movement is the only justification for supposing that life is extinct and not in abeyance.   Similarly in *Astacus* we find that the eosinophile cell is very much more stable than the hyaline cell.   Correlated with this fact we find that the change of environment due to the presence of bacterial poisons will call forth the most active manifestation of the special functions of the eosinophile cell, while the hyaline cell may be paralysed or destroyed.   It is possible, however, that the poisons of all micro-organisms do not possess this discriminating value.   It may be that if a sufficiently large number of micro-organisms were taken, they could be placed in a series commencing with those which acted towards the cells like indifferent particles, being ingested by the hyaline cells and unnoticed by the eosinophile cells, and ending with those like *B. anthracis* or *filamentosus*, which provokes the most profound activity of the eosinophile cell while the hyaline cell is incapable of attacking them.   Bearing in mind the complex conditions of the conflict, we are forced to conclude that the organisms against which the Frog is not immune may be found either at one end or at the other end of the series, or at both.

Yeast, in its most virulent form, represents to a certain extent the middle of the series.   Clusters of yeast cells are attacked by the eosinophile cells, just as are anthrax chains, and to these come hyaline cells ; in fact, the story is the same as that which has already been related.   At the same time hyaline cells will ingest stray yeast cells which have not been attacked by eosinophile cells.   The notes of one experiment will illustrate this.

Experiment VII.

Bouillon yeast culture of great virulence when tested on Rabbits.   Inoculated lymph drop with this. Frogs completely unharmed by large doses of this yeast.

1 *o'clock.*—Watched large mass of yeast.   Three eosinophile cells came up and were lost in north end of mass.

Saw hyaline cell ingest stray torula.

1.12.—The same hyaline cell has now reached north end of mass and is attacking it.   After three minutes it is lost in mass.

1.25.—Another eosinophile cell appeared and became lost in south end of mass.

1.30.—Another eosinophile cell, followed by hyaline cell, came in at north end and both fused with mass.

All this time the eosinophile cells have been covering the mass all over.

1.35.—At north end yeast has lost much of its distinctness.   After some hours plasmodium formed, as with anthrax.

9 *o'clock.*—One eosinophile cell left plasmodium.

9.10.—Same cell came back and apparently fused with another.

9.15—Another eosinophile cell left.

Discontinued observation, and therefore did not see the general breaking up.   Stained the preparation, and found many rose-reacting cells.

The hyaline cell is mostly, but not always, capable of digesting the yeast cells,

which it ingests. Similarly it can digest some, but not all the anthrax spores which it ingests, and we may classify the activities of the hyaline cells under two heads :—

(1.) The ingestion of particles which they can digest, such as coagulated proteid, dead micro-organisms, and certain living micro-organisms or spores. These the hyaline cells eliminate from the body in which they dwell by dissolving them.

(2.) The ingestion of particles which they cannot digest, such as Indian ink, or dust particles. These they do not eliminate from the body, but carry them to the connective tissues and there hide them away. Instances of this are found in the removal of dust particles by the phagocytes of the lung, and in the fate of Indian ink when injected into the body of *Dytiscus*, as shown by DURHAM.*

SECTION VII.

The Rose-reacting Cells.

It would appear, from the facts so far set forth, that the fate of the microbe in the Frog's lymph depends upon the activity of two kinds of cellular elements. Are we to attribute, therefore, no part in the conflict to the non-amœboid cells which are charged with rose-staining granules? This question is at once answered by the fact that one of the conditions apparently necessary to a successful issue to the conflict between the eosinophile cells and the micro-organisms, whether the conflict occur in the body or out of the body, is an increase in the number, and still more in the size and granulation of these cells. In the lymph of newly-captured, healthy Frogs, the rose-staining cells are usually very small, and so few in number, that they would be completely overlooked were it not for the brilliant rose tint which their granules take with methylene-blue. This condition is shown in figs. 4 and 5A.

The injection of micro-organisms is followed by a continuous increase in the number of these cells, and they now appear as shown in fig. 5B. The increase is a striking fact from the second to the fortieth hour. Beyond that time we have not followed them.

To state the same fact in other words, the rose-staining cells are continuously abstracting some substance or substances from the plasma and depositing it within themselves as rose-staining (basophile) granules. For the present we are content to suggest that the substances abstracted are foreign, abnormal substances in solution, such as, for instance, the poisonous products of bacterial activity. Frequently, in watching the processes taking place in a drop-culture, one sees the eosinophile cells at first vigorously attacking the bacteria, and then the whole process will come, sometimes almost abruptly, to a standstill, the eosinophile cells contracting and becoming spherical, while the hyaline cells disintegrate. This, we explain, by supposing that the bacterial poison in small doses stimulates the eosinophile cells, while in larger

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* DURHAM, "On Wandering Cells in Echinoderms," 'Quart. Journ. Micros. Sci.,' vol. 33.

quantity it paralyses and ultimately kills them.   If this be true, it is obvious that in the body some mechanism or mechanisms must exist for keeping the amount of bacterial poisons either taken in at once, or produced by the as yet unattacked or unkilled bacilli, below the limit at which it is fatal to the wandering cells or to the body at large.   We suggest that this function is, in part, filled by the rose-reacting cells.

The evidence bearing on this question which we have so far obtained may be briefly summarised as follows.

The presence in the plasma of foreign bodies in solution, such as egg albumen or anthrax albumose, leads to an increase in size, number, and granulation of the rose-staining cells.   This is true both of the Frog and of *Astacus*.   Also, as has already been stated, if *Daphnia* is exposed to toxic substances, an increased formation of a rose-reacting (amphophile, in this case) substance results.

The alteration in the chemical composition of the plasma of a hanging drop of lymph due to clotting also leads to a similar result.   Lastly, we may cite the absence, often noted by us, of rose-reacting cells in lymph in which the bacilli have grown, and this in spite of the fact that these cells are very resistent to the toxic substances.   The presence pointed out by SHERRINGTON of similar cells in large numbers round the intestinal blood-vessels in cases of enteric disease suggests that they are there to intercept the toxic substances streaming in from the lumen of the intestines.   The increase in the number of these cells in inflammation, in carcinoma, &c., and, generally speaking, in cases normal or pathological, in which there is an abnormal production of normal or abnormal products of metabolism was noticed by KORYBUTT, DASKIEWICZ, and EHRLICH.[*]

The importance attached to the removal of the bacterial poisons, and the endeavour made by the body to get rid of them, are strikingly shown by *Daphnia* and *Astacus*.

In *Daphnia* the whole blood stream may be watched on the stage of the microscope.   The presence of bacterial poisons in the body is then seen to increase the adhesiveness of the corpuscles ; they tend to adhere to the walls of the blood spaces, but they are mainly attracted to the excretory organs (the shell glands) around which they cluster in large masses.   At the same time the epithelium of the excretory organs becomes more granular, vacuoles appear, and the inner surface of the cells becomes irregular, being pushed out into processes.

In *Astacus* the injection of very large quantities of *Bacillus filamentosus* into the pericardial sinus is followed by the almost complete disappearance of the filaments from the blood in half an hour.   Drops of blood taken from the pericardial sinus, and from the ventral sinuses, both abdominal and thoracic, were stained and examined, but prolonged searching failed to reveal more than isolated rods or pairs of rods, and these only at the rarest intervals.

[*] EHRLICH, "Beiträge zur Kenntniss d. granulirten Zellen, &c.," *loc. cit.*

Finally, the animal was bled into some iodine solution, and the cells allowed to sink to the bottom. After twelve hours the sediment was examined, and only one or two cases of eosinophile cells attacking bacilli were found. The bacilli themselves were almost absent. The walls of the blood spaces were then examined and the missing bacilli discovered embedded in plasmodial masses clustering round the green glands, the excretory organs of *Astacus*.

It may be said in passing that the eosinophile attack appeared to precisely resemble that observed in the FROG's lymph.

SECTION VIII.

Preliminary Observations on Mammals (Rabbit and Rat).

(1.) The peritoneal fluid of these animals is full of cellular elements, these being, just as in the Frog, chiefly eosinophile and hyaline cells. On killing the animal (by decapitation), and quickly placing a little of the fluid on a small drop of a bouillon culture of anthrax on a warm stage, the initial attack by the eosinophile cells at once takes place. The latter, in the case of the Rat, is pronounced and rapid; while in the Rabbit, which is a more susceptible animal, it also takes place, but is less vigorous. It was impossible, with the method employed, to observe more than the initial stage, on account of the difficulty experienced in keeping the cells alive for longer than fifteen minutes. However, the eosinophile cells deported themselves in exactly the same manner as they do in the Frog, moving towards the chain of bacilli, and fusing along it.

(2.) The following experiments and observations, made on Rabbits, will throw some light on the mode of action of the eosinophile cells :—

LEBER has demonstrated the powerfully solvent action of pus on such substances as copper, gold, and silver, &c. It has been shown that the cellular elements of pure and fresh pus consist of practically nothing else than cells with fine or coarse eosinophile granulation. On repeating some of LEBER's experiments, and placing minute pieces of sterilized copper, steel, and silver wire into the anterior chamber of a Rabbit's eye, under strict aseptic and antiseptic precautions, in all cases a suppuration rapidly ensued, most sudden when copper was used. This pus invariably contained nothing but granular cells. Already, after 24 to 48 hours, the copper was found to be roughened and corroded. We conclude from the observations that the solvent action of pus is due to the eosinophile granulation.

Cellulose, in the shape of sterilized cotton-wool, placed in the anterior chamber under similar precautions, was not affected in the slightest, even after an interval of six weeks. Cellulose is both an extremely indifferent substance and also most resistant.

(3.) Others have already demonstrated the presence of a ferment in pus. Ross-

BACH, before us, succeeded in separating an amylolytic ferment from the leucocytes ('Deutsch. Med. Woch.,' 1890), while LEBER ('Die Entstehung der Entzündung') found that pus digested fibrin and liquefied gelatine.

Summary.

Some of the phenomena described and discussed in §§ II. to VII. may be summarized as follows :—

(1.) The three different kinds of wandering cells, the eosinophile cell, the hyaline or non-granular cell, and the basophile rose-reacting cell, proliferate while free in the body fluids. This may be demonstrated in the Frog, and has, in the case of other animals, been recorded by ourselves and other workers.

(2.) The different kinds of cells multiply independently, so that the numbers of any one kind of cell may vary without a corresponding variation in the numbers of the other cells. There are thus three kinds of leucocytosis, corresponding to the three forms of wandering cells found in lymph or peritoneal fluid.

(3.) The three kinds of cells are differently affected by different substances when introduced into the plasma.

(*a*.) Solid substances of the nature of what are commonly called indifferent substances affect only the hyaline cells which ingest the particle. Coagulated proteid and Indian ink are examples, as are also anthrax spores.[*]

(*b*.) Anthrax and filamentosus bacilli, when first introduced, attract only the eosinophile cells, which kill or maim them by means of a substance derived from their stored eosinophile granules. After the bacilli have been thus acted on they can become the prey of the ingestive hyaline cells.

(*c*.) Vermilion and yeast cells stand midway between indifferent substances and these bacilli, and attract both hyaline cells and eosinophile cells. Vermilion only slightly attracts the eosinophile cells. Yeast cells attract them strongly, but also to a certain extent are immediately ingested by the hyaline cells.

(*d*.) The rose-reacting cells are increased in number and size by alteration in the chemical composition of the plasma, such as is produced by clotting, or by the introduction of toxic albumose or egg albumen. Hence they are probably chiefly active in maintaining the normal constitution of the plasma so far as dissolved substances are concerned.

(4.) The eosinophile cells are highly specialized bodies endowed with the power of movement, in virtue of the possession of a pseudopodial ectosarc, and with glandular powers directed to the production of a bactericidal, or at least antibiotic, substance.

SECTION IX.

The facts brought forward in the preceding sections lead to certain conclusions as to the morphological position and physiological attributes to be accorded to the sporadic mesoblast.

Morphology.

In the Frog, the sporadic mesoblast consists of three kinds of cells, the eosinophile cell, the hyaline cell, and the rose-reacting cell, together with the red corpuscles and platelets. Of the two latter, we, personally, can say nothing from our own knowledge ; nor have we any reason beyond the general facts of their distribution in the body, for placing them in the same morphological group with the three first-named elements.* Leaving then the red corpuscles and platelets out of account, we find that the sporadic mesoblast of the Frog contains elements precisely similar to those which compose the similar tissue of such a widely divergent animal as *Astacus*. The large phagocyte, derived from the hyaline cell and of temporary existence only, is not only found in both animals, but produced by essentially similar conditions in both. This exact resemblance between animal forms so diverse is helpful to us, since it gives us good reason for supposing that the three cell elements of the Frog have arisen by the differentiation of a primitive homogeneous sporadic mesoblast, that is, one the cell elements of which are all alike.

This question of the comparative morphology of the sporadic mesoblast may, like other morphological questions, be approached in two ways. We may investigate the phylogenetical position of the tissue or its ontogenetical position. In both directions our knowledge is very incomplete. No conclusions as to lines of development of the tissue within the limits of the Chordata can be based on our present knowledge, for in the lowest member of the Chordata which we have examined, the *Ammocœte* larva, all three types of cells are already present. At the same time, so far as we know, there is no exact description of the histology of the wandering cells of the Tunicata or Cephalocorda. In the Crustacea, the conception of the origin of the different wandering cell types from an archetype finds strong phylogenetic support. The group of the Crustacea is remarkable for including within its limits animals of the very simplest and animals of the most complex organization. The very simple animal *Daphnia* has a sporadic mesoblast composed of only one kind of cell, *Astacus*, on the other hand, representing the most complex members of the group, has three kinds. But the evidence does not rest here. The wandering cells of *Daphnia* are not only of one kind, but also this one cell performs all the functions, and has all the morpho-

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* ZIEGLER, "Die Entstehung des Blutes," 'Ber. d. Naturforsch. Gesellsch. zu Freiburg,' vol. 4, H. 5, as the result of his investigations on the development of the blood, arrives at the conclusion that the red corpuscles are morphologically distinct from the white corpuscles. The former are of intravascular origin, the latter are extravascular at first, but migrate into the blood system.

logical characters of the three cells of *Astacus*. This fact has already been discussed by one of us in earlier papers,[*] but we are able to add a further archaic character of the cell to those given there. In the earlier papers, the specific granulation was justly described as rose-reacting, further investigation has revealed the fact that the rose-reacting substance of *Daphnia* is also amphophile. In other words, even in its specific granulation the wandering cell of *Daphnia* is archaic, it is a rose-reacting, amphophile granulation. We may even pursue the search for the cell element of the undifferentiated sporadic mesoblast a stage further back to the wandering cell of the larval *Daphnia*, while still within the broad pouch of the mother. Then we find an amœboid cell with no granules, but the whole cell substance reacts with aniline dyes, giving "a faint but distinct rose-reaction with methylene-blue."

Since Tadpoles are not available in summer or autumn, we have not as yet been able to pursue the ontogenetical study of the corpuscles of the Frog. Fœtal Cats, however, are to be found in all seasons, and in these animals we have so far found only one kind of wandering cell which is without specific granulation, and has a spherical nucleus and scanty cell substance. In the very late fœtus the wandering cells still show no granulation but there are now two kinds of cells present, one with a round nucleus and one with a lobed nucleus. In the adult all these three types are undoubtedly present. Thus we find a certain phylogenetic and ontogenetic support for the statement that the very divergent elements of the sporadic mesoblast have a morphological homogeneity in the fact that they have arisen from a primitive amœboid wandering cell with no specific granulation, by a process of morphological and physiological differentiation akin to that which has, *pari passu*, led to the increasing complexity of the animal generally.

Physiological.

The question of the functional significance to be attributed to the sporadic mesoblast also finds a partial solution in the very incomplete series of facts we possess bearing on the physiology of this structure. Undoubtedly the wandering cells are present largely as a protective mechanism to guard against the intrusion of foreign substances, living or non-living, into the organism. But this is probably only a small part, and not the most primitive of their functions. They are also related to the general processes of the body, notably to the bodily nutrition. In *Daphnia* they may be readily watched engaged in the transportation of fatty particles from the alimentary canal to its place of storage.[†] Also any abnormal condition of the perivisceral fluid, or blood, leads to the massing of these cells around the special excretory organs of this animal, and this fact suggests that their activities are partly directed to maintaining the normal constitution of the body fluids so far as the dissolved matters are concerned.

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[*] 'Journ. of Physiology,' vol. 13, p. 184, *seq*., and p. 318.

[†] 'Journ. of Physiology,' vol. 13, p. 184, *seq*.

Thus, though exact knowledge is wanting, yet it is abundantly clear that the homogeneous sporadic mesoblast of *Daphnia* is intimately related to the processes of general physiological importance taking place in the alimentary canal and the excretory organs; and there is no sufficient reason to lead us to believe that this connection has been entirely, or to any great extent, lost in the course of the divergence and specialization of these cells, which has produced the different wandering cell-types of the higher animals. On the contrary, it is a matter of common physiological knowledge that the wandering cells are profoundly affected by events occurring in the alimentary canal. Thus, though we are not able to point to any particular physiological process carried out by these cells, still we have sufficient grounds for opposing the supposition that they exist only to protect the body from the invasion of foreign particles, and that appears to us to be a valuable step and a most necessary preliminary to a successful study of their functions.

In endeavouring to indicate the lines along which the specialization of the sporadic mesoblast has advanced, probably in most animal groups, we may proceed with perhaps greater sureness. The primitive mobile, ingestive, and glandular cell of *Daphnia* has become in *Astacus* the specially glandular eosinophile cell, the specially mobile and ingestive hyaline cell, and the specially absorptive rose-reacting cell, and what we know of the sporadic mesoblast of the Vertebrates points to its having developed along similar lines.

But both older and later observations* on the part played by wandering cells in the formation of scar tissue, indicate that an even more extended conception of the relations of the sporadic mesoblast may at some future time become necessary, and the convenient fiction, wherein the blood was regarded as a tissue, like cartilage or connective tissue, only with a fluid matrix, may yet be found to embody a morphological truth.

The Relation of the Attack of the Eosinophile Cell to the Ingestive Act of the Hyaline Cell.—A study of the way in which some of the carnivorous Protozoa capture and ingest their prey, throws a clear light on the relations of the peculiar mode in which the eosinophile cell attacks a bacillus to the simple ingestive act of a hyaline cell, or *Amœba*. Further, if all these facts are placed together, they suggest many thoughts on the relation of intra-cellular to extra-cellular digestion.

By comparing the various accounts given by LEIDY, M. GREENWOOD, and other observers, of the manner in which an animal like *Amœba* or *Actinosphærium* captures and ingests its prey, we find that the following processes may be recognized:—(1) Contact is effected with the prey and its movements are arrested; (2) then, after it has thus been maimed or killed, the prey is ingested and digested. But the captured infusorian may resist the benumbing influence of the captor, and may, after being exposed for a long time to the *extra-cellular* attack, as it were, acquire tolerance of it,

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* Compare SHERRINGTON and BALLANCE " On Formation of Scar-tissue."   ' Journ. of Physiology,' vol. 10, p. 550.

recover all its powers and escape. The best instances of the killing of prey as a result of mere contact, are furnished by the Suctoria. When a moving animalcule comes into contact with the long stiff, very specialized pseudopodia of these animals, its movements are suddenly arrested. The phenomenon is so striking that GRÜBER, MAUPAS, and others, speak of it as a poisoning of the prey by some substance excreted by the captor.

Expressing these facts in terms of the processes occurring in the cell, we have

(1.) The contact of the prey stimulating the captor to *excrete* a poison ;

(2.) The ingestion of the now inert body ;

(3.) The *secretion* of a digestive fluid which dissolves the ingested prey.

LEIDY's account of the capture of a *Urocentrum* by an *Amœba*, clearly shows how essentially similar the excretion of the poison is to the secretion of the digestive fluid. His words are, " a second victim of the same kind was included in the fork of a pair of pseudopods, the ends of which were brought into contact so as to imprison the animal in a circle. The latter moved restlessly about within its prison but, after a time, became motionless, and shortly after the ends of the pseudopods, which enclosed it fused together . . . and finally ⸱the *Urocentrum* was enclosed."* If we now turn to M. GREENWOOD's† carefully detailed account of the phenomena of ingestion in *Amœba,* and notice the close morphological relation between the space "included in the fork of a pair of pseudopods" and the digestive vacuole, the primitive unity of the two processes cannot fail to be seen. In the very specialized Suctoria, the excretion of the poison and the killing of the prey is a specialized and much more perfect process, as is also the very remarkable manner of its ingestion.

Turning now to the eosinophile and hyaline cells, we see that in the former the *extra-cellular* act has become its special character, and, as in the Suctoria, the perfection of the mechanism for the production of the poison has shortened the latent period of its discharge. The cell is, in short, a unicellular gland, *but* it preserves intact the initial step of the primitive process, it effects contact with its prey; the hyaline cell also effects contact with its prey, but the extra-cellular discharge is insignificant or absent, the ingestive and *intra-cellular* act being, on the other hand, complete and rapid.

We have strictly parallel phenomena in the endodermal cells, and this enables us to advance the line of thought a step further. METSCHNIKOFF‡ has given us good reasons for supposing that the primitive endoderm, or nutritive cell, of the Metazoa, carried out its functions in a manner exactly resembling the *Amœba* described by LEIDY. But in the Cœlenterates we find an endoderm composed of (1) a gland-cell which forms *extra-cellularly* a digestive fluid, (2) an ingestive cell which produces *intra-cellularly* a digestive fluid, and (3) an absorbent cell which is correlated with the gland cell and takes up the fluid products of the extra-cellular digestive process.

* LEIDY, "Rhizopods of North America." See the figs. 5 and 38 on Plate 1.

† "Journal of Physiology," vols. 7 and 8. "On the Digestive Process in some Rhizopods."

‡ METSCHNIKOFF, 'Zool. Anz.,' 1878, p. 387.

Viewed in this way the eosinophile cell is analogous, in its activities, to the gland cell of the Cœlenterate endoderm, and, for instance, to the cells lining the fundus of a gastric gland of the Mammalia.*

Other instances of an extra-cellular digestive act are found in :—

(1.) The attack of *Vampyrella spirogyræ* and *V. pendula* on the Algæ which furnish them with food. This furnishes a most interesting parallel.

(2.) The digestion of fibrin by the surface cells of the mesenterial filaments as described by KRUKENBERG.†

(3.) The hollowing-out of spaces in the first bone spicules by the osteoclasts.

Lastly, we are not without some feeling of the bearing of our observations on the most difficult question of immunity. But further knowledge has only increased our perception of the complexity of the problem. We see that it depends upon all the possible permutations and combinations of the activities of the three wandering cell elements engaged in the conflict, and of that yet entirely unknown factor, the general physiological reaction of the organism to the microbic poisons. In the face of these difficulties we have deemed it wise to stifle the temptation to theorise on the subject, until, with wider knowledge, a greater capacity to cope with the difficulties shall have been attained.

DESCRIPTION OF FIGURES.

PLATE 29.

Fig. 1. A group of two hyaline cells and one eosinophile cell fixed with ·25 per cent. iodine as rapidly as possible after removal from a subcutaneous lymph sac of a Frog. The hyaline cells are larger than usual. The vertical line represents the relative length of the major axis of a red corpuscle. Oc. 10, Ob. apochr. ⅛th POWELL and LEALAND. Camera lucida.

Fig. 2. Eosinophile cell fixed by heat and stained with eosin and methylene-blue. From lymph of healthy Frog. Same magnification as fig. 1. Camera lucida.

Fig. 3. Eosinophile cell and a small hyaline cell. Normal Frog. Fixed and stained with methylene-blue solution. Oc. 4, Ob. E ZEISS. Camera lucida.

Fig. 4. Rose-reacting cell and hyaline cell of normal Frog. Fixed and stained with methylene-blue solution. Viewed with artificial light. Oc. 4, Ob. E ZEISS. Camera lucida.

Fig. 5. Rose-reacting cells (*a*) of normal Frog, (*b*) 12 hours after injection of yeast, from the seat of the inoculation. Stained only with methylene-blue.

* Compare M. GREENWOOD, "Digestion in Hydra," 'Journ. of Physiology,' vol. 9.

† KRUKENBERG, "Ueber d. Verdauungsmodus d. Actinien," 'Vergleichend-Physiol. Studien,' Heidelberg, 1881.

Illuminated with yellow light.   Same Oculars and Objectives as in fig. 1. Vertical line = length of major axis of red corpuscle.

Fig.   6. Cells from blood of late fœtus of Cat.   Fixed with heat and stained with eosin and methylene-blue.   (*a*) and (*b*) different forms of white corpuscle, of which (*b*) is much more abundant, (*c*) nucleated red corpuscle—very few present.   The vertical line represents the diameter of the non-nucleated red corpuscle.   Oc. 10, Ob. apochr. ⅛th Powell and Lealand. Camera lucida.

Fig.   7. Hyaline cell budding.   From hanging drop inoculated with anthrax.

Fig.   8. Young hyaline cell showing active movements, but unable to effect contact with anthrax chain.   From same field of microscope as fig. 7.

Fig.   9. Hyaline cell dividing.   Leucocytosis produced by injection of urari.   Acidulated solution of methyl-green.   Oc. 4, Ob. E Zeiss.   Camera lucida.

Fig. 10. Eosinophile cell in neighbourhood of anthrax chain.

Fig. 10b. The same cell shortly after it has effected contact with the chain.

Figs. 11, 11a, 11b, 11c, 11d, 11e.   Successive stages in the attack of the cells on a chain of anthrax bacilli in a hanging drop of lymph.   11e drawn with camera lucida.   Oc. 4, Ob. D Zeiss.   Illustrating Experiment I.   See p. 300.

Fig. 12. A hyaline cell in the same field as fig. 11e.   Not drawn to the same scale. The two sketches illustrate successive phases of this cell's movements.

Fig. 13. An eosinophile cell just about to attack the end of a chain of *B. filamentosus.*

Fig. 14. Eosinophile cell which has just attacked an anthrax chain.   Fixed and stained with the methyl-blue solution.

Fig. 15. An eosinophile cell with granules completely discharged, attacking anthrax chains.   Fixed and stained with the methylene-blue solution.

Figs. 16, 16a, 16b, 16c, 16d. Successive stages of conflict with a chain of anthrax, which in fig. 16 is already hidden in the cell mass.   Illustrating Experiment II.   See p. 302.

Fig. 17. The amphophile condition of the eosinophile cells, also an eosinophile cell which has completely lost its granules, and a hyaline cell.   See Experiment VI., p. 307:

Fig. 18. A hyaline cell and an eosinophile cell.   In the same field of the microscope. See Experiment III., p. 303.

1

2

5

a

b

6

a

b

c

3

7

8

4

10b

9

13

anthrax
chain

11a

10

14

16

11b

15

11c

12

a

11e

16a

a

b

16b

11d

18

a

16c

16d
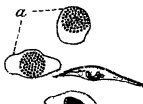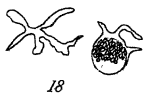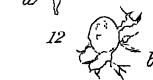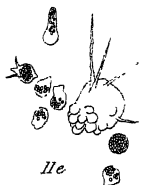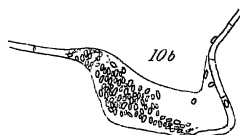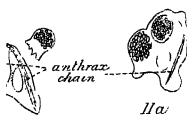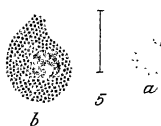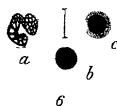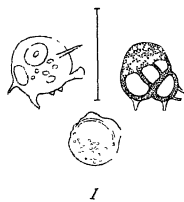
VIII. *The Process of Secretion in the Skin of the Common Eel.*

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[PLATES 30–33.]

2.5.94

INTRODUCTION.

WE owe to LEYDIG (1) the first demonstration that a secretory process is a possibility for the skin of the Fish. In some twelve genera he described the occurrence of specialized cells in the epidermis, to which he gave the common name of "Schleim-zellen," though subsequent research has shown that several varieties of structure were included in this generic term.

KÖLLIKER (2) in 1860, in an investigation of the skins of *Myxine* and *Petromyzon*, differentiated two cell-forms. Observing the "thread bodies" of the mucous sacs of *Myxine*, first clearly described by JOHANNES MÜLLER (3), he recognized their cellular nature, and found that the epidermis held similar, though less complex cells, from whose contents also, by appropriate treatment, a thread could be obtained. To these epidermic cells of *Myxine* the name "Fadenzellen" was applied. In addition, clear or finely granular cells, termed "Schleimzellen," were described.

The cells in the epidermis of *Petromyzon*, considered as the analogues of the "Schleimzellen" of *Myxine*, were described as "keulenförmige," with a vesicular space at the swollen end holding a pair of nuclei, and with elongated neck, "mit zarten, häufig leicht wellenförmig gebogenen Längsstreifen versehen." A second special form was taken as corresponding with the "Fadenzell." Globular in shape, but supplied with a long thread-like process, it appeared to be filled with granules, which, however, were considered by KÖLLIKER to be the optical expression of a closely-wound thread included in the cell. The name "Körnerzellen" was given to these structures. A secretory function was considered probable for the "Schleim-zellen" of both *Myxine* and *Petromyzon*, but there are no direct observations upon the specific point.

A year later, MAX SCHULTZE (4) reinvestigated the structure of the skin of the same two Fish.

In the case of *Petromyzon* he renamed KÖLLIKER's "Schleimzellen," "Kolbenför-mige Gebilde" or "Kolben," emphasized the occurrence of longitudinal striation in these structures, but also added a transverse striation of the neck, insisted upon as not superficial, but produced by alternating discs of isotropous and anisotropous material—an appearance, however, absent in the fresh specimen. These "Kolben" were described as always in contact with the corium by their lower ends, and at this point a connection with a nerve filament was considered as possible. Indeed, MAX SCHULTZE looked upon these structures as of the nature of nervous end-organs, possibly

contractile, on account of the points of similarity to striated muscle fibre presented by the neck viewed by polarized light.

Similar structures were also briefly described for the epidermis of the Eel and Tench.

MAX SCHULTZE further showed that these "Kolben" are the analogues of the "Fadenzellen" of *Myxine*, and not of the "Schleimzellen," as KÖLLIKER supposed, the "Körnerzellen" being identical with the latter.

HEINRICH MÜLLER (5), in 1864, was the first to observe in *Petromyzon planeri* that the "club cells" (Kolben) do not necessarily retain their connection with the corium, as MAX SCHULTZE stated. From observations of the variations in number, shape, and position in the epidermis, he supposed that these cells are liable to extrusion. Thus (p. 44): "Es findet diess in der Art statt, dass es sehr nahe liegt, eine *Abstossung der Gebilde mit oder ohne Wiedersatz* anzunehmen" (*italics* in original). He did not, however, actually observe this extrusion. He expresses himself as opposed to the assumption of a nervous connection, and considers these structures as modified epithelial cells of uncertain function.

The work of KÖLLIKER, MAX SCHULTZE, and H. MÜLLER had been chiefly upon the three species of *Petromyzon*, and, in the case of the two former observers, upon *Myxine* also.

F. E. SCHULZE (6), in 1867, extended our knowledge of the epidermis of Fish by an investigation of fifteen genera, including *Petromyzon*. Three forms of specialized cells—one of which, however, was found peculiar to *Petromyzon*—were described.

(1.) "Becherzellen," found in all the genera taken, characterised by possession of a distinct "theca," filled in the fresh state with granules imbedded in a more fluid mass, and whose secretory activity was actually observed and figured in the case of the barbel of *Cobitis fossilis* (Plate 7, fig. 3), and seen also in the skin of the caudal fin and head of small Eels. An account is, moreover, given of an Eel dropped upon a sandy floor, relieving itself of the casing of adherent grit, in the form of a tube with slimy interior, after replacement in water.

(2.) "Kolben," seen only in *Tinca, Leuciscus, Cobitis, Anguilla, Esox, Silurus*, and *Petromyzon*.

(LEYDIG (1) had previously investigated the first five of these genera, and, since he gave no special description of "club cells," must have included both "Kolben" and "Becherzellen" under the one term "Schleimzellen.")

In dealing with the important point as to whether the "club cells" are permanently fixed to the cutis or capable of passage to the surface, F. E. SCHULZE describes them as breaking away from the cutis in all cases except in the Lamprey and Eel. (He figures, however, a case in the Eel in Plate 7, fig. 7, but considers it pathological.) In the Tench the "club cells" near the surface become irregularly rounded or flattened, and the possibility of their extrusion is mentioned. Thus, p. 160: "Solche veränderte Kolben habe ich vielfach dicht unter der äussersten Zellenlage gefunden, so dass wohl

kein Zweifel darüber bestehen kann, dass sie beim Ausfallen einer darüberliegenden Zelle selbst auf die Oberfläche des Fisches gelangen."

F. E. Schulze considered the "club cells" as secretory in nature, and as possibly forming a sebaceous matter (Talgdrüsen). He could not substantiate Max Schultze's statement regarding the presence of transverse striation in the neck.

(3.) The "Körnerzellen" of the Lamprey were more closely described by F. E. Schulze, their processes being considered as due to tubular prolongations of a cell membrane, yet as passing within the body of the cell to become united by a junction piece resembling the head of a pair of compasses. They were considered to be sense cells, but no nerve connection was demonstrated.

Langerhans (7), in his monograph on *Petromyzon planeri*, failed to observe the "club cells" clear of the corium. The most interesting point, however, in his description of the skin of this Fish, is the recognition of a form of cell not hitherto described. Under the name "kleine Rundzellen," pp. 16 and 17, he notes cells resembling lymphocytes as occurring between the other elements of the epidermis. These are figured in Plate 1, fig. 11, as of from 4μ to 5μ in diameter, and possessed of scanty protoplasm. He considers that these cells represent chromatophores which have not developed pigment.

To A. Fœttinger (8) (1876) is due a valuable paper upon the epidermis of *Petromyzon*. The most important point in connection with this paper is the complete corroboration of Heinrich Müller's statement as regards solution of continuity between the "club cells" and the corium, and, further, the actual demonstration that these bodies escape from the epidermis, and may be found lying upon its surface (Plate 2, figs. 8, 9, 10, 11, 12; Plate 3, fig. 37). Thus, p. 629: "la massue entière ou tout au moins la partie qui se colore en jaune par le picrocarminate d'ammoniaque est eliminée." The process of extrusion was carefully followed.

A vacuolation between the surface of the club and an enveloping membrane is said to occur, loosening it by the formation of a network of granular material (Plate 2, fig. 1). An accumulation of fluid in the spaces of this network, especially at its lower part, is considered to force the "club cell" upwards, leaving its membrane *in situ*. In the passage out the nuclei disappear, and are always absent from the extruded cell.

He agrees with F. E. Schulze as regards the structure of the goblet cells, but does not agree with this observer as regards the nervous nature of the "Körnerzellen."

The characters of the goblet cells common to the epidermis of all Fish have been especially described by List (9), who, while describing the "footed" and "footless" forms of F. E. Schulze, further subdivides the latter into "stalked" and "un-stalked" varieties, the stalk being merely a prolongation of the theca of the cell.

List differentiates the contents of the theca into a "filar mass" arranged as a net and staining easily, and a homogeneous "interfilar mass" in the meshes of the former. He considers that the contents of the theca are extruded, when the goblet

reaches the surface by a turgor of the interfilar material as the result of imbibition of water.

LEYDIG (10) in a later paper has given a few more details of interest in connection with this communication.

In the lower layers of the epidermis of two Batrachian larvæ (*Pelobates fuscus* and *Hyla arborea*), he describes and figures cells holding much convoluted threads, which occasionally pass beyond the border of the cell. He compares these to the "Faden-zellen" of *Myxine*, and terms them "Byssuszellen." EBERTH (11) had previously described very similar cells in the epidermis of the larva of *Bombinator igneus* (see especially Plate 25, figs. 19 and 24, of his paper).

LEYDIG admits the membrane described by FŒTTINGER for the "club cells," and ascribes MAX SCHULTZE's transverse striation to folds of this structure (Plate 7, fig. 7). He notes, too, in the "club cells" of *Petromyzon marinus* an appearance of tangled threads. He considers both the "club cells" and "Körnerzellen" of *Petromyzon* as secretory in nature.

FRITSCH (12), in his account of the epidermis of *Malapterurus*, describes four varieties of cells : (*a*) "Kolbenzellen," (*b*) "Becherzellen," (*c*) "gewöhnliche Epider-miszellen," and (*d*) "Kornzellen."

He considers the "club cells" to be secretory in function, and in his figure (Plate 8, fig. 25) indicates a point where these cells have escaped from the epidermis, and also "club cells," the contents of whose "vacuoles" have been discharged.

The goblet cells arise, he thinks, from the ordinary epidermic cells of the middle layers. The ordinary epidermic cells are characterised by the possession of a "vesicular" nucleus which is poor in chromatin, and in strong contrast to that of the fourth variety, viz., the "Kornzellen." These "Kornzellen," which are evidently the "kleine Rundzellen" of LANGERHANS (7), are very similar to ordinary lympho-cytes. LIST (13) has figured similar cells in the epidermis of *Cobitis fossilis*, and considers that they are of the nature of the wandering cells described by STOHR (14) in the surface epithelium of the tonsil.

FRITSCH makes no very definite statement of opinion as regards the origin and fate of these cells. In his description of Plate 8, fig. 25, he marks them *Leucocyten ?* yet in the text he expresses himself as opposed to LIST's hypothesis that they are wandering cells in the ordinary sense of the term, since he failed, in contradistinction to the latter observer, to find these cells between the fibres of the corium.

FRITSCH did not see any evidences of division of these cells, though LIST figures forms which he takes to represent such a process.

On the whole, he inclines to the rather curious view that they supply the most superficial epidermic scales.

Still more recently POGOJEFF (15), who appears to have been ignorant of the work of FŒTTINGER, has written on the structure of the epidermis of *Petromyzon*. By the use of gold chloride he sees a violet staining fibril within each "club cell" connected

with two cells, the whole being surrounded by endothelial sheaths after the manner of a Pacinian corpuscle. He thus agrees with the old idea of MAX SCHULTZE, that the "club cells" represent the end apparatus of cutaneous nerves, and considers that his failure to observe solution of continuity between the lower ends of the "club cells" and the corium supports this view. He strangely enough maintains that the statement that the "club cells" leave the corium has arisen from observation of oblique sections! He agrees with KÖLLIKER in considering the "Körnerzellen" of the epidermis of this Fish to be of the nature of unicellular glands.

It will be evident from the above brief account of the work of previous observers that an anatomical basis for a secretory process of considerable complexity and peculiar nature exists in the epidermis of many Fish. The skin, apart from its protective and sensory functions, which are not here under consideration, may also fairly be considered as a glandular surface of special construction, and the absence of localised collections of secretory cells provided with ducts such as we find in higher Vertebrates is no basis for the statement occasionally met with that "the skins of Fishes are non-glandular." Unicellular secreting structures whose function it is to supply the slime bedewing the surface of the animal are abundant in the epidermis, and in the special case of the Myxinoid Fishes these unicellular structures actually become collected to form glands in the commonly accepted sense of the term.

In none of the above researches is any mention made of the payment of special attention to the condition of secretory activity of the skin at the moment that the specimen was fixed for histological purpose. To this is to be attributed to a large extent the divergence of opinion concerning the functions of the "club cells" in particular. Nor, except in the well-known case of *Myxine* (32), has the fresh slime of the Fish been subjected to the microscope.

Chance stimulation, due to handling in capture, varying in amount in different cases, must have accelerated the normal secretory process more in some cases than in others, and produced a change in the histological picture and the physiological deduction therefrom. In no case, as far as I am aware, has direct experimental excitation been employed.

I propose in the following pages to give an account of the secretory process in the skin of the common Eel, deduced, in the first place, from the histological appearances at various levels of the epidermis and the formed elements of the slime, and, in the second place, from those changes that can be artificially produced by experimental irritation.

Special care has been bestowed upon the Fish in order that the condition of the skin at the time of preservation might be known.

The animals were always caught without a hook, and were kept some days after caturre in running water and supplied with food.

To obtain a skin in the *lowest phase of secretory action* hybernating sluggish Fish were employed, and death was effected by transfixion of the medulla. If this is skilfully performed the animal instantly becomes motionless, and pieces of skin may

be removed without reflex movement (and concomitant reflex secretory action) during the period of persistence of the condition of " shock."

A Fish that struggles, from failure to strike the right place at once, becomes a "sliming" Fish, and is useless for a picture of the slow process of normal secretion, though useful for that of some of the higher phases of the act.

The methods of artificial stimulation will be found under their appropriate headings, and the histological procedure in the Appendix.

§ 1. FORMED ELEMENTS OF THE SLIME OF THE EEL.

Seeing that the secretory structures of the skins of Fish are not provided with ducts, there is no method of escape for the secreted material, except by actual casting off of the superficial epidermic scales. The slime, therefore, always contains numbers of epidermic scales, unavoidably thrown off in the process of its formation.

For purpose of collection of the secreted matters, three methods have been employed—(1) Placing the animal in a bath of pilocarpine solution; (2) Subjecting the animal to faradization; (3) exposing the Eel to the vapour of chloroform. By the first and second methods it is possible to collect little "flocks" of secreted matter as they rise from the surface of the Fish; in the third method, the secretory activity is very energetic, and the passage of a blunt scalpel gently over the surface of the Fish removes abundant material.

Apart from the epidermic scales above mentioned, the following formed elements are present in the secretion collected by any of the above methods : (a) *Threads;* (b) *Nuclei;* (c) *Granules;* (d) *Goblet cells.*

(a.) *Threads of the Slime.*—These structures occur singly in much convoluted masses (Plate 31, fig. 14), or more extended, but generally in the form of masses of more finely fibrillated material (Plate 30, fig. 1). The extraordinarily tenacious character of the slime is undoubtedly associated with the presence of these threads.

The most pronounced case of a thread secretion in Vertebrates is, of course, the well-known instance of the Myxinoid Fishes, where the threads are known to arise by a metamorphosis of the specialized " Kolben," contained in the ventral slime glands (KÖLLIKER (2), MAX SCHULTZE (4), also BLOMFIELD (16)).

Thread-like structures within epidermic cells are also described, by EBERTH (11), in the lower layer of the epidermis of the larva of *Bombinator igneus*, and, by LEYDIG (10), in the epidermis of the larva of *Pelobates fuscus* and *Hyla arborea*, though neither observer noted their escape in a secretion. An able account of many ectodermic thread secretions in Invertebrates and their possible relation to those found in Vertebrates has been given by EISIG (17), from which it is evident that such threads may be used for the construction of protective tubes (*Cereanthus*, certain Annelids, Insect larvæ), for catching prey (*Holothuria nigra*, Spiders), or for attachment to a fixed object (Byssus of Lamellibranchiate Molluscs). The thread of the

Sea Stickleback (*Gasterosteus spinachia*), used for nest building, has been shown, by Möbius (18), to be a secretion of the renal epithelium.

The use of the threads in the secretion of the Eel appears to be to ensure that the slime shall adhere to any object with which it is brought in contact.

The threads have the following characters. The breadth of the coarsest may be from 2 μ to 3 μ, but all conditions of finer fibrillation are found, the finest fibrils being beyond accurate measurement.

Usually present in wavy masses of the finest fibrils entangling other elements, one often, especially when the pilocarpine method is used, comes across convoluted masses of the coarser variety. The extremities are often wound in a corkscrew fashion (Plate 30, fig. 1, on the right).

They are unchanged by induction shocks applied to fresh material. Do not swell in water. Readily give the xantho-proteic reaction. Resist digestion with pepsin and hydrochloric acid, and with trypsin and sodic carbonate. One per cent. sodic hydrate leaves them unaffected. Osmic acid darkens them slightly. Treated with picro-carmine, they stain brilliant yellow (Plate 30, fig. 1).

They are thus composed of some albuminoïd material of very resistant nature, possibly allied to keratin.

(b.) *Nuclei of the Slime.*—Free nuclei are frequent in the fresh slime; they vary in diameter from 2 μ to 4·5 μ, and are generally spherical, but occasionally ovoid (Plate 30, fig. 1).

No distinct chromatin network or filament was seen, though these nuclei take most dyes easily. In no case could a cell body be distinguished, though, at times, a group was met with surrounded by a flocculent material.

(c.) *Granules of the Slime.*—Small spherical granules (·75 μ to ·5 μ) abound in the fresh slime, and can be preserved upon cover-glass preparations. They present the following characters.

They give the xantho-proteic reaction. Are soluble in five per cent. acetic acid. Swell in one per cent. sodic hydrate, but do not dissolve. Do not dissolve easily in water (still clearly outlined in slime kept a day in water). Are not soluble in five per cent. sodic chloride solution containing five per cent. of acetic acid. Resist peptic and tryptic digestion. Are present on cover-glass preparations, after treatment with boiling alcohol and ether. In cover-glass preparations treated with corrosive sublimate they do not give the reddish-violet colour with thionin, considered, by Hoyer (19), to be characteristic of mucin. They take the methyl green of the Biondi stain, prepared according to Heidenhain's (20) recipe. They also stain easily with soluble blue and carmine.*

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* January, 1894. Since writing the above I have found that these granules stain deep brown when treated on a cover-glass with nitro-molybdic solution, followed by pyrogallol, according to the method of Linossier and Moury (33) for micro-chemical detection of phosphorus. They are possibly, there-fore, of the nature of nucleo-albumin.

(*d.*) *Goblet cells.*—Extruded goblet cells occur in the slime, though rather rarely. Their characteristics will be entered into below in connection with the elements of the epidermis itself.

The epidermic scales found in the slime are flattened scales, devoid of "prickles"; the cell body, punctated with fine dark granules, measures from 15 μ × 10 μ up to 23 μ × 12 μ; the nucleus is large and vesicular, and measures from 5 μ × 4 μ to 12 μ × 6 μ. There is no evidence of any mucinous change in the protoplasm of these cells, and the cell bodies refuse to give the reddish-violet reaction with thionin.

All the above elements of the slime are seen in Plate 30, fig. 1.

In addition, however, mucin is undoubtedly present in "solution" in the slime, for dilute alkaline extracts give a precipitate with acetic acid, insoluble in excess, and upon long boiling with two per cent. H_3SO_4, a substance reducing FEHLING'S fluid is formed. Furthermore, in cover-glass preparations, one gets streaks and nets of a material distinguishable from the threads by taking the carmine instead of the picric acid of picro-carmine, staining reddish-violet with thionin, and also giving the orange-red colour with saffranine, described, by PANETH (21), as characteristic of mucin.

The slime is alkaline to litmus, but, curiously, will not affect phenolphthalein, a point which I have also noticed with *Myxine* (37).

§ 2. The Structure of the Epidermis.

Before attempting to account for the origin of the elements of the slime just described, it is necessary to detail the structure of the epidermis. In this account I shall describe the epidermis as it appears in its lowest phase of secretory activity as prepared by the method mentioned on pp. 324 and 325, and this condition will be spoken of as "normal" in contrast to the condition evoked by artificial excitation, which will be designated "stimulated."

The structure of the normal epidermis has been investigated by the two methods of teasing and section-cutting, and will be described under two appropriate headings.

(α.) *Teased Preparations.*

Fresh teased preparations are not successful, the tenacious slimy nature of the epidermis not allowing sufficient separation of the elements. I have therefore resorted to maceration in RANVIER'S "third part" alcohol, with subsequent staining, and teasing in dilute glycerine, or making cover-glass preparations of the macerated material with subsequent staining.

(1.) *The club cells.*—These curious cells are always the most conspicuous element in teased preparations. The great variety of form and size of these bodies, indicating that they are subject to continual metamorphosis in the process of their development, will be evident from a glance at some of the forms shown in Plate 31.

A medium stage in development will be the most convenient point from which to commence description.

In such condition the club cells are pyriform with much elongated stalk, the total cell being from 50 μ to 100 μ in length, the greatest breadth 15 μ to 30 μ, tapering below to a blunt end varying from 2 μ to 10 μ across. The stalk is beset with small projections, giving it a "shaggy" appearance under the high power.

Within the upper expanded part of the club is seen a vesicle, bordered by a lattice work of clear material in stout struts, and filled with finely granular matter. This vesicle is as a rule ovoid, its longer axis coinciding with that of the club and measuring from 12 μ to 13 μ in the long, and 9 μ to 20 μ in the short diameter. One nucleus with distinct nucleolus is always present, resting against the wall of the vesicle, but two such nuclei often occur (figs. 15a and b, Plate 31). At the lower end of the vesicle the protoplasm of the stalk of the club presents a central core of material more granular than the peripheral parts, and this core may extend for variable distances into the stalk, often reaching nearly to the lower end.

It is not however necessary to find one vesicle only, two such may be present (figs. 16a and 16b, Plate 31), and in such cases each holds a nucleus.

As regards action of dyes, the most marked reaction is that with picro-carmine, in which the material of the stalk and walls of the vesicle stains brilliant yellow (similar to the threads of the slime), while the nucleus stains red, the contents of the vesicle usually remaining colourless. This reaction has already been noted by FŒTTINGER (8) for the club cells of *Petromyzon*. With the Biondi mixture, the body of the club takes the acid fuchsin, the contents of the vesicle and the nucleus the methyl green.

Among these comparatively simple club cells are found others which evidently represent further stages in development.

Apparently two somewhat different processes may occur, though in the end both lead to the same nett result, viz., the production from the cell body of a convoluted or spiral fibre ("fibre-mass") which is capable of subsequent further division into fibrils, and the extrusion of the contents of the vesicle in the process of development of the fibre.

In both processes the final result is obtained by the vacuolation and canalization of the material of the upper part of the body of the club, proceeding generally from below upwards and from the regions in the immediate proximity of the periphery of the vesicle towards the surface.

The variation in the process above alluded to, lies in this, that an incomplete shell of the original club body material may be left adherent round the vesicle, so that when set free it appears as a cell with nucleus and cell wall composed of the club cell body material (formation of "escape cell"), or, on the other hand, the vacuolation may be more complete and so free the contents of the vesicle with little adherent matter (formation of "escape mass").

The first process, viz., the formation of an "escape cell," is illustrated in figs. 17a, b,

and c, Plate 31. In 17b it is evident that the superficial lattice work round the original vesicle comes away with it as an outer coat to the "escape cell." It would appear probable that this lattice work is the result of the erosion of the club body material immediately in contact with the wall of the vesicle, in the process of vacuolation that leads up to its escape.

Cells bearing such a superficial lattice-work-like thickening of the cell membrane have been described in the epidermis of certain larval Amphibians, and are generally known as "LEYDIG's cells." Originally described by LEYDIG (22) as "Schleimzellen" in the larva of *Proteus* and *Salamander*, the description in the latter animal was subsequently supplemented by LANGERHANS (23), FLEMMING (24), PFITZNER (25), and LIST (9). LIST described them also in the larva of *Triton*, and CARRIÈRE (26), and PAULICKI (27) in *Siredon pisciformis*, the latter observer naming them "Netzzellen." LEYDIG (28) too has more recently again given a description for the cells in larval *Salamander* (see especially his fig. 25, Plate 31).

The rib-like thickenings of the cell membrane were seen by PFITZNER in the living epidermis, and are not, therefore, due to a precipitation by reagents as FLEMMING supposed. FLEMMING and PAULICKI, moreover, showed that the nucleus of these cells is not necessarily "incised" as LANGERHANS originally described. The fate and function of the cells in larval Amphibians is quite unknown. They at any rate, however, belong exclusively to the period of aquatic life (PFITZNER and PAULICKI), and during that period undergo division (FLEMMING, PFITZNER, and PAULICKI).

These "escape cells" are frequently found isolated in teased preparations of the Eel's skin, and in many cases bear a striking resemblance to the figures of the LEYDIG's cells of larval Amphibians given by the above authors.

Figs. 21, 22, and 23, Plate 31, are illustrative of the structure of these cells, and are all drawn to the same scale. The size is very variable, $65\,\mu \times 50\,\mu$ to $20\,\mu \times 12\,\mu$, the majority, however, having an average size of $40\,\mu \times 30\,\mu$. The lattice work takes the picric acid of picro-carmine, suggesting that it is of the same composition as the club cell body material, while the granular contents stain faintly pink. The larger specimens (figs. 21a and b) generally bear attached a convoluted piece of the "thread mass," to be shortly described. Occasionally these cells bear distinct traces of the method by which they have been shelled out by a process of vacuolation, bearing attached some of the bars of the lattice originally formed in the substance of the head of the club (figs. 22a, b, and c). Two nuclei are often present (figs. 22b, 23a), though whether this is due to origin from the vesicle of a bi-nuclear club or to subsequent division is not clear, though nuclei with two nucleoli (fig. 23b) would seem to suggest the latter as a possibility. I have never seen any mitotic figures, however, in these nuclei.

In other cases the nucleus is found on the point of being extruded (figs. 23b, c, and d).

In the second process of development of the club cell, viz., the production of an

" escape mass," the vacuolation of the material of the club head appears to be more extensive, and, instead of a special capsule remaining round the original vesicle, the whole is broken up, leading to setting free of the granular mass within. Such granular masses ("escape masses"), often still holding the original nucleus of the vesicle, are frequent in the teased preparations, and are evidently only a modification of the escape cells above described.

The process of vacuolation of the club head is illustrated in figs. 20*a*, *b*, and *c*, Plate 31, and it is at once evident from a glance at these how the " fibre mass," consisting of the remains of the club body material is formed by being " gutted out " of the head of the club, the stalk remaining attached.

A splendid example of an isolated " fibre mass " is figured in fig. 20*d*, Plate 31. It is to be noted that these fibre masses are always coiled or spirally wound (figs. 18*a* and *b*, 19*a* and *b*, 20*c* and *d*), suggestive of an " elater" action in removal of the surface epidermic scales to allow of escape of secretion ; I have not, however, been able to convince myself of any hygroscopic property.

The " thread mass " thus formed is capable of further disintegration. Secondary vacuolation is often seen within it at its upper part (fig. 19*a*, Plate 31),. and it is finally possible for it to be split into the finest fibrils similar to those found in the secretion (fig. 4*b*, Plate 30). These finely fibrillated fibre masses stain brilliant yellow with picro-carmine, and there can be no doubt that the fibres of the secretion are ultimately derived from the fibre masses of the club cells.

The earliest stages in the development of the club cells will be more conveniently considered in connection with the description of the sections.

In addition to the club cells the other cell forms found in teased preparations merit a short description here.

These are—(ii.) *Ordinary epidermic cells ;* (iii.) *Goblet cells ;* (iv.) *Connective tissue cells,* (*a*.) Pigmented, (*b*.) Unpigmented ; (v.) Small round cells to which the name of "*fibroblasts*" will be given.

(ii.) *Ordinary epidermic cells.*—These vary much in shape and size according to the depth in the epidermis from which they are taken (as shown by comparison with sections). Illustrations of the various forms will be found in fig. 26, Plate 32.

Cells coming from the region between the stalks of the club cells are laterally compressed with much elongated nuclei (*a*, *b*, *c*, and *l*), while the more superficial cells are polyhedral or rounded with large rounded nuclei (*h* and *i*). It is only upon cells of the latter shape that " prickles" are distinct. Bi-nucleated cells are frequent, but here again I have failed to find mitotic figures (*f, g, j,* and *k*).[*]

* January, 1894. In *Petromyzon*, F. E. SCHULZE (6) figures (taf. 8, fig. 2*b*) one of the surface epidermic cells with porous cuticular border, in a stage of transformation into a goblet cell, and W. B. HARDY (35) has described a secretion of granules from the same cells in the Ammocœte larva. In *Myxine* I find several rows of secreting goblets at the surface. In the Eel, as will be evident from the plates and as I have found by treating the fish with dilute methylene blue solution, there is no evidence of a secretory process in the surface cells of the epidermis.

(iii.) *Goblet cells.*—The full account of these will be found in the description of sections. The cells in teased preparations from macerations in RANVIER's alcohol are usually swollen and ruptured, presenting an empty theca with wide or narrow stoma, some remnants of protoplasm holding the nucleus lying at the base (fig. 27, Plate 32).

(iv.) *Connective tissue cells.*—That cells of mesoblastic origin may find their way into the epidermis of certain animals is well known. LEYDIG (29) first drew attention to the presence of pigmented connective tissue cells in the epidermis of Reptiles, Fish, and Annulates (*Piscicola*). F. E. SCHULZE (6) described such structures in the epidermis of Eel, Tench, Sturgeon, Silurus, and Ruff. LEYDIG (30) again has shown the presence of such cells among the epidermic cells of Gasteropods, and later (10) demonstrated that "Strahlenzellen," *quite devoid of pigment*, could also occur in the epidermis of Fish (*Cyprinus carassius*). In the same paper he figures (fig. 32, Plate 9) contractile chromatophores from the epidermis of *Pelobates fuscus*. ZIMMERMANN (31) too has described the occurrence of chromatophores among the epidermic cells of the Frog and larval Salamander and their division (mitotic) in the latter.

The pigmented connective tissue cells (chromatophores) of the epidermis of the Eel send their much ramified processes among the inter-epithelial spaces, and are evidently capable of locomotion, since in sections one finds them at various levels, and present in varying number at different spots. They are extremely rare in the epidermis of hybernating specimens. Since these cells break easily in teasing, one only sees their form to advantage in sections, and those figured in fig. 24, Plate 32, are from such preparations.

The non-pigmented connective tissue cells, which LEYDIG has shown may exist among the epidermic cells of Fish, are very abundant in the case of the Eel ; indeed, a regular network of these cells appears to exist in the lower layers of the epidermis.

The processes of these cells are of extreme fineness and great length in many cases, and it is these processes that form a felt work surrounding especially the stalks of the club cells.

Between these fully developed, non pigmented connective tissue cells and the fifth kind of cell to be described (fibroblast) a complete series of forms can easily be traced (see fig. 25, Plate 32).

(v.) *Fibroblasts.*—The cells to which I have given this name are without doubt identical with the small rounded cells resembling lymphocytes described by LANGER-HANS (7), LIST (13), and FRITSCH (12), already alluded to in the introduction to this paper. The total cell measures only 4 μ to 5 μ, has a nucleus extremely rich in chromatin, and a barely distinguishable rind of protoplasm externally. A few of the isolated cells are figured in the upper left-hand corner of fig. 25. These cells are frequently found dividing, and mitotic figures are easily recognized in their nuclei. As mentioned above, transitional forms between these cells and the unpigmented connective tissue cells are readily found, the change being produced by a simple growth of the protoplasm out into processes, usually commencing on one side first.

The richness in chromatin of the nuclei of these fibroblasts is one of their most marked features, and the nuclei of the developed connective tissue cells have the same characteristic, so that they are very conspicuous in sections (see figs. 5 and 13, Plate 30 ; also Photo E, Plate 33). The origin of these cells will be considered in the description of sections.

(β.) *Sections of Normal Epidermis.*

It is only in sections that the origin of the elements differentiated in teased preparations can be adequately traced. The sections have been cut in planes both vertical and parallel to the surface of the skin. That the sections in the former direction were not oblique was judged at once by observing the appearance of the conical palisade cells of the lowest layer.

The elements requiring description are :—

> (*a*.) The palisade cells and their immediate descendants.
> (*b*.) The club cells at various levels.
> (*c*.) The goblet cells.
> (*d*.) The fibroblasts.
> (*e*.) The formed connective tissue elements.

(*α*.) *The Palisade Cells.*

Situated upon a basement membrane of some two or three micro-millimetres in thickness, separating the epidermis from the corium, is a single layer of conical cells, whose apices are often prolonged for some distance between the cells of the super-jacent layers. The height of these cells is very variable (8 μ to 35 μ) ; the basal breadth is from 4 μ to 8 μ (see figs. 2, 5, 8, &c., Plate 30 ; also Photo A, Plate 33).

The body of the cell stains brownish-yellow with FLEMMING's fluid, the externally directed process being usually more deeply tinted.

The nuclei are oval, with long axis coinciding with that of the cell, " vesicular," with distinct nuclear membrane, and, as a rule, rather poor in chromatin. In the resting state these nuclei measure 3 μ to 12 μ in length and 1·5 μ to 4 μ in breadth.

Evidence of nuclear division is common (fig. 7, Plate 30), but here again I have never seen mitotic figures. As regards the plane of division of the cell, it appears to be more often parallel to the long axis of the cell than transverse, so that dividing nuclei are most clearly seen in sections cut parallel to the surface of the skin. Divisions at right angles to the long axis are, however, not infrequent.

The descendants of these cells are: (1) *the club cells ;* (2) *the goblet cells ;* (3) *the ordinary epidermic cells.*

(1.) *Origin of Club Cells from Palisade Cells.*—In transverse sections across the long axis of the palisade cells it is often evident that a lateral bulging of the cell

body occurs before division takes place. This lateral enlargement differs in staining reaction and appearance from the rest of the cell body. In material hardened in FLEMMING's fluid and stained with saffranine, this differentiated portion appears homogeneous and stained brownish-yellow, while the rest of the cell body is faintly granular, and either lighter yellow or stained pink by the saffranine (see fig. 7, Plate 30).

This lateral enlargement forms the body of the young club cell, and having received its nucleus from the dividing palisade cell, it commences to grow towards the surface.

As a consequence of this mode of origin, one often finds in vertical sections of early stages that the slender lower end of the young club cell is wedged in between two palisade cells, and still in contact with the basement membrane (see fig. 2, *a, b, c,* and *f,* Plate 30). Though the method of origin above described is by far the most common, it is also possible for club cells to arise by the method of division of the palisade cells at right angles to their long axis (see again fig. 2, *e, g,* and *h,* Plate 30). In this case the pointed process of the palisade cell grows out, and at the same time undergoes the same change as occurs in the formation of the lateral enlargement in the first method, becoming homogeneous and staining browner than the rest of the cell body with FLEMMING's fluid.

The further development of the club cells will be followed under (*b*) of this section.

(2.) *Origin of Goblet Cells from the Palisade Cells.*—A young goblet is always distinguishable from a young club cell in this, that the upper part of the cell instead of being homogeneous and brown in FLEMMING's fluid and saffranine preparations, is finely granulated and stained red. In fact, the transformation of some of the protoplasm into mucigen occurs before the cell has been constricted off from the palisade cell that gives it birth (fig. 9*a,* 1, 2, and 4, Plate 30).

The nucleus too, in this early stage, shows a far more distinct nucleolus as a rule than that of the young club cell, and this large nucleolus is a feature in the nucleus of the fully developed goblet cell. In the origin of a goblet from a palisade cell, the rule is for the palisade cell to divide at right angles to its long axis, but origin by division parallel to the long axis also occurs. Both of these methods are illustrated in figs. 9*a* and *b,* Plate 30.

The method of origin of the goblet cells of the Fish epidermis has, I find, been variously described by previous observers.

F. E. SCHULZE (6), who investigated thirteen Teleostean genera, including the Eel, and also *Accipenser* and *Petromyzon,* considered that the goblet cells arise from the ordinary epidermic cells of the upper and middle layers, and, in *Petromyzon,* he figures (fig. 2B, Plate 8) a surface cell becoming transformed into a goblet. FŒTTINGER (8) confirms SCHULZE's account for *Petromyzon,* and FRITSCH (12), using *Malapterurus,* also agrees with SCHULZE with reference to the origin from the ordinary epidermic cells. LIST (9, III.), however, in *Cobitis* and *Torpedo* saw the goblets arising in the lowest layers, though he does not exclude the possibility of origin from the upper cells also.

In the Eel, I have never seen any other method of origin than that above described, *i.e.*, direct from the palisade cells, and these small closed goblets can be traced up through the layers above, expanding as they came to maturity, and finally discharging at the surface.

(3.) *Origin of the ordinary Epidermic Cells from the Palisade Cells.*—Dividing palisade cells are often met with, which show neither of the modifications above described.   There can be little doubt that the descendants of these are the ordinary epidermic cells, seeing that cells of the character of those found nearer the surface, but elongated from compression between the stalks of the clubs, are found low down, immediately above the palisade cells (see figs. 29 and 30, Plate 32 ; also fig. 9*b*, 7, Plate 30).

Again, at the edge of the lip, club cells are absent and goblets few, the mass of the epidermis being formed of the ordinary epidermic cells (see fig. 32, Plate 32).

(b.) The Club Cells at Various Levels.

The young club cell once separated from its mother palisade cell rapidly grows up into the layers above, and undergoes the following process of development :—

A vesicle becomes developed in the neighbourhood of the nucleus.   This process may commence even before the club has separated from the palisade layer (see *g* in fig. 2, Plate 30), but the rule is that the vesicle does not develope till later.

The formation of the vesicle is preceded by the development of a marked granularity in the material immediately surrounding the nucleus (fig. 3, Plate 30).   This granular material stains as densely with saffranine as the nucleus itself, though, whether it represents extruded nuclear material or not, I am unable to state definitely, though I am inclined to consider it as modified cell body material.   The contents of the vesicle are formed from this granular material, and, as the vesicle grows in size, it is easy to see at its borders, in many instances, a layer of the granular material.

As regards the orientation of the vesicle, it may appear at the outer or inner side of the nucleus, or at both simultaneously (fig 28, Plate 32).   A distinct nucleolus is visible within the nucleus at this stage.   The material within this vesicle is different to the rest of the club body, and, moreover, does not agree in reaction with the material formed in the goblet cells.   The reasons for this statement are as follows : the material in the vesicle stains a grey colour with FLEMMING's fluid, which is in marked contrast to the brownish-yellow, or brown colour, taken by the material of the club body (figs. 5 and 8, Plate 30).   In sublimate specimens treated with the Biondi stain, the material of the vesicle takes the methyl green, while the club body takes the acid fuchsin.   Again, as regards differences between the contents of the club vesicles and those of the goblet cells.   In specimens fixed with FLEMMING's fluid, saffranine stains the goblets but not the club vesicles (figs. 5 and 8, Plate 30) ; the same is true of methylene blue.   With thionin, the goblets stain reddish-violet, but the club vesicles are not stained (fig. 13, Plate 30).   In specimens treated with FLEMMING's fluid, soluble blue is the

only stain with which I have succeeded in staining the contents of the club vesicles (fig. 6a, Plate 30). In sublimate or picric acid specimens, however, saffranine, hæmatoxylin, and methyl green will all dye this material.*

The formed vesicle now continues to enlarge, and a curved line is often noticeable across its wall, separating a denser part below from a less dense part above (fig. 8, Plate 30). This is probably related to the differentiation of a membrane for the escape cell, since in later stages the vesicle again looks homogeneous.

The surrounding wall of club cell body material soon now becomes thinned on one side (fig. 6b, Plate 30), and either an "escape cell" is distinctly set freeam ongst the upper epidermic cells (fig. 30, Plate 32), or an "escape mass," containing more or less of the club body material in its walls, is found above the remaining fibre mass (fig. 13, Plate 30 ; Photo A, Plate 33).

During these later stages the contents of the vesicle assume a distinctly granular appearance in Flemming hardened material, which is absent in the younger condition (cf. figs. 6a and b, Plate 30), and the "escape mass" holds even more granular contents, which, however, retain the peculiarity of staining with soluble blue (fig. 6c, Plate 30).

Sections through the upper layers of the epidermis, parallel to the surface, show the escape masses with their granular contents and wall of club body material remarkably clearly. It will be noticeable from a glance at fig. 6b, Plate 30, and fig. 30, Plate 33, that the substance constituting what I have termed the "fibre mass," darkens with the osmic acid of the FLEMMING's fluid to a greater extent than the rest of the club body material, and even in the young club cells the apex and neighbouring parts is, at a very early stage, found to be gradually becoming differentiated in this manner, the process extending from the outer towards the inner part.

Finally, the "escape masses" or "cells" come to lie immediately beneath the surface scales, and are extruded, breaking up in the process, for they are never found as such in the slime itself.

As regards the final fate of the "fibre mass," that part of it which does not pass out with an escape mass, may remain for some time in the epidermis (fig. 5, Plate 30), but it also is gradually eliminated, and one often comes across gaps between the surface cells lined by a few fibres (fig. 13, Plate 30, and fig. 33, Plate 32), indicative of the point of extrusion. Whether the fibre mass is merely passively extruded by the pressure of surrounding growing cells, or whether by virtue of its spiral structure, aided by hygroscopic property, it actively takes part in its own elimination, I cannot say for certain, but the appearance presented in fig. 29, Plate 32, and certain considerations in connection with the histological appearances in artificially stimulated skins, would seem to be in favour of the latter hypothesis.

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* January, 1894. I find that the contents of the vesicles of the club cells readily give the LILIENFELD and MONTI (33) phosphorus reaction. The material in the goblet cells, on the other hand, remains unstained, obviously on account of the absence of phosphorus from mucin.

(c.) *The Goblet Cells.*

The mode of origin of these cells has been considered in § *a* (2). Once free of the palisade cells they appear to be pushed up by the new cells originating below, growing in size as they approach the surface, but remaining completely closed till it is reached. In material hardened in FLEMMING's fluid the nucleus which lies in a protoplasmic appendix of the theca (LIST's "befusste Becherzellen") always holds a remarkably large and distinct nucleolus (figs. 8, 9, 12, and 13, Plate 30, also Photo A, Plate 33). The youngest separated goblets measure 8 μ to 10 μ, the oldest surface specimens may be as large as 60 μ. The shape is very variable (figs. 9, 11, and 12, Plate 30) on account of the variable pressure to which they are subjected at different levels in the epidermis.

The contents of the theca appears in the form of distinct granules in the younger goblets if fixed with FLEMMING's fluid or osmic vapour, but in sublimate and picric acid specimens and even in FLEMMING's fluid material in the large surface cells one sees the appearance described by LIST (9, III.) of a "filar and interfilar mass."

Since I have only seen this latter condition in the surface cells in osmic vapour and Flemming hardened material, I consider that it is produced by a process of imbibition of water by the contents of the theca when it nears the surface. I agree with LIST in considering that some such imbibitory process accompanied by swelling brings about the rupture of the theca, and one frequently finds the surface scales in the act of being forced up by an expanding mass of extruded mucus, and later stages in which a plug of mucus projects from the stoma (figs. 12*b* and *c*, Plate 30) above the general surface of the epidermis. The staining reactions of the contents of the theca at all stages of development have already been referred to; the thionin reaction (figs. 11 and 13, Plate 30) is perhaps the most noteworthy. In no case have I seen a goblet cell with two nuclei, a fact confirmed by LIST (9, III.) in the case of the epidermis of Fish.

Apparently the life of a goblet cell is not ended when it reaches the surface and discharges its first load of mucus. Forms are often found in which the protoplasmic foot appears to be growing up (fig. 12, Plate 30, *c* and *d*, on the right), and again elongated cells filled with granules staining black with osmic acid and devoid of mucigen (fig. 11, Plate 30, on the left), which appear to represent the protoplasmic "foot" of a goblet cell. Furthermore large rounded cells, whose body stains reddish-violet with thionin, while the nucleus stains blue, are sometimes found two or three cells below the surface (fig. 10, Plate 30). I can only regard these cells as regenerating goblet cells, and agree with LIST that in the Fish epidermis, at any rate, a goblet cell is capable of more than one discharge of mucus.

(*d.*) *The Fibroblasts.*

These small round cells, already referred to in the description of the teased preparations, are a very marked element in sections on account of the intensity with

which their nuclei stain with most dyes. They are most numerous in the intervals between the apices of the palisade cells, where they often occur in little masses. As one passes towards the surface the relative quantity of these cells diminishes, and in the lower phases of secretory action I have not found them amongst the upper epidermic structures, though in stimulated Fish it may be otherwise. The nuclei in the lower collections of these cells very frequently present mitotic figures (fig. 5, Plate 30), and there can be no doubt that local multiplication occurs.

As regards the origin of these cells, I agree with LIST (13) that they are wandering cells, for not only do I find exactly similar cells between the fibres of the corium as he did in *Cobitis*, but I note them passing through the basement membrane and between the palisade cells, as is very evident in Photo E, Plate 33. The function and fate of these cells is considered in the next paragraph.

(*e.*) *The Formed Connective Tissue Elements.*

These, as already stated on p. 331, are of two kinds, pigmented and non-pigmented.

The pigment cells require no description here as they have already been referred to under (α) of this section, p. 331. They may reach very near to the surface as is evident in fig. 10, Plate 30, but their number is very variable in different specimens.

The non-pigmented connective tissue cells are difficult to distinguish in well-preserved specimens where no shrinkage has occurred ; one, at most, sees a fibrous appearance between the other elements, especially in the lower layers of the epidermis. If, however, one observes sections of material in which the club cells have undergone shrinkage (this often occurs in picric acid hardened skins) a complete supporting net formed of the branches of connective tissue cells becomes evident. Photo F, Plate 33, is from a section taken parallel to the surface and the network is there evident. See also fig. 31*b*, Plate 32.

These connective tissue cells appear to arise from the small wandering cells, for, as already mentioned and figured in fig. 25, Plate 32, all stages can be traced between the two forms. I can see no evidence whatever for FRITSCH'S hypothesis that they supply the superficial epidermic scales, indeed the demonstration in Photo F, Plate 33, that these cells are of extra-epidermic origin removes the ground from under it at once. Nor again do I see any evidence in the Eel in support of LIST's hypothesis that these cells undergo any degenerative process and pass out as " Schleimkörperchen " in the secretion.   Normally, I am convinced that they form the epidermic supporting tissue, and I therefore see no need here of considering any phagocytic theory that might possibly be advanced. I shall again have to refer to these cells in connection with the consideration of the effects of artificial stimulation.

Addendum to § 2.

Nervous structures and connections.—Nerve fibres passing from corium to epidermis are easily observed, and on the afferent side the "becherförmige Sinnesorgane,"

originally described by LEYDIG (1, 10, 29, p. 84), are often met with, being especially clear in the lip (fig. 33, Plate 32). As regards, however, a connection between the secretory elements and the nervous system, I have no certain anatomical results to describe ; even in gold chloride preparations I have not been able to convince myself.[*] As will be seen later, however, in connection with the effects of stimulation, there appears to be physiological evidence of such connections.

Variations in distribution of secreting elements.—F. E. SCHULZE (6) observed that the club cells do not exist in *Petromyzon* at the edge of the ventral fin, nor in the lips. In the lip of the Eel I find they are quite absent at the edge (fig. 32, Plate 32) and on the inner surface, though as one passes towards the general surface of the head these cells gradually make their appearance. The edges of the pectoral fins too are devoid of club cells, though they are present upon the rest of the surface of these organs. The goblet cells I find present over the whole surface of the animal, though local increases in their relative amount (and the same is true of the club cells) are often noticeable. The goblets are most numerous on the inner surface of the lip.

§ 3. The Origin of the Formed Elements of the Slime.

Having now considered the structure of the epidermis and the mode of development of its elements, it is possible to treat of the origin of the elements of the slime, whose consideration was discontinued at p. 327.

Threads.—That the threads of the slime originate from the " thread masses" of the club cells there can be no doubt.

It has been seen that elongated " thread masses" on the point of escape (fig. 5, Plate 30) can be detected in the epidermis, and that surface gaps lined by fibres (fig. 13, Plate 30, and fig. 33, Plate 32) may also exist. Again the threads of the slime, and the bodies and "thread masses" of the club cells, give the same brilliant yellow reaction with picro-carmine. And finally, in teased preparations from the epidermis of stimulated Eels one often finds "thread masses" distinctly breaking up into threads (figs. 4a and b, Plate 30).

The club cells then of the epidermis of the Eel are " Fadenzellen" in the sense applied by KÖLLIKER (2) thirty-three years ago to those of the epidermis of *Myxine*, though in the case before us we have no specialized glandular involutions of the epidermis in which the process of thread formation is carried out to its full extent.

The unravelled threads would not easily be differentiated from unwound threads of *Myxine*, and such a specimen as that depicted in fig. 14, Plate 31, is suggestive even of the complexity of the " aufgewickelte Fadenkörper" of JOHANNES MÜLLER from the slime glands of *Myxine*.

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[*] January, 1894. A few preparations were made by the " Golgi method," but, unfortunately, only the "slow process" was employed, and the delicate epidermis was not well preserved by the potassic bichromate without addition of osmic acid. I intend to again investigate the point by the aid of the "rapid method" with osmic acid, which, I believe, will preserve the integrity of the structure.

In this connection I may state that from *Petromyzon fluviatilis* by suitable stimulation I have obtained a thread secretion which is undoubtedly formed from the club cells of the epidermis, and the elements of which give the characteristic reaction with picro-carmine. FŒTTINGER (8) saw the extrusion of the club cells in this Fish, but he does not describe the final stage of thread formation.

I am, therefore, quite unable to accept the more recent account by POGOJEFF (15), as regards the nervous nature of the club cells of *Petromyzon*, and conclude that, in the three cases I have examined, *Myxine, Anguilla,* and *Petromyzon*, the greater part of the club cell is eliminated in the forms of threads, which can be detected in the slime.

Nuclei.—As regards the nuclei of the slime, two possible origins present themselves, (1) From the nuclei of the vesicles of the club cells ; (2) From the nuclei of the fibroblasts.

Size is, unfortunately, no help in coming to a decision between these two possible sources of origin, though it at once enables one to state that these slime nuclei are not those of epidermic scales, for the latter are far larger than the former.

FŒTTINGER (8) noted in *Petromyzon* that the extruded club cells did not contain a nucleus, and the fact is evident enough in the fibre masses of the cells in the Eel. The nucleus of the original club cell passes away in the escape cell, or mass, and as this is absent as such from the slime, it must break up upon extrusion, and its nucleus be set free.

As regards the other possibility, origin from fibroblasts, I have not observed that, under "normal" conditions, these reach the surface. On the whole, then, I am inclined to consider the slime nuclei as derived from those of the club cells, though, under conditions of severe stimulation, I think an origin from fibroblasts is also possible.

· *Granules.*—The granules of the slime appear to be the granules of the escape cells, or masses, developed from the contents of the vesicles of the club cells. Their general similarity, and the staining reaction with soluble blue and methyl green, together with the similarity of the LILIENFELD and MONTI phosphorus reaction (*v.* footnotes pp. 326 and 345), in the two cases, form, however, the only basis upon which I make this statement. The mucin streaks and nets found in cover-glass preparations of the slime must owe their origin to the extruded contents of the goblet cells.*

§ 4. THE HISTOLOGICAL APPEARANCES IN ARTIFICIALLY STIMULATED SKINS.

The details of the process of secretion, so far deduced mainly from the examination of the slime and the variations in appearance of the elements of the "normal" skin, are confirmed, and to some extent supplemented, when the skins of animals submitted to artificial stimulation are investigated.

Summer Fish were used, as a rule, since these are quicker to react to irritation than

* January, 1894. I have failed to find evidence of the presence of any proteolytic ferment, similar to that extracted by Miss ALCOCK (36), from the skin of the Ammocœte larva, in the skin of the Eel.

those obtained in winter, and stimulated skins were obtained in three ways, (1) The pithed animal was suspended in a vessel filled with the vapour of chloroform ; (2) The pithed animal was subjected to faradization ; (3) An animal, not rendered motionless by the first act of pithing, was allowed to writhe and slime ("mechanical stimulation").

Of these three methods, the first is by far the most efficacious for the production of the highest phase of excitation ; indeed, the secretory changes within the epidermis are almost "volcanic" in character, and the structure becomes so loosened that successful histological work requires great care. Since, however, a narcotic action of the chloroform vapour on the elements of the epidermis must finally supervene, this method is not so suitable for observation of the effects of prolonged stimulation as the second. In the third method, one generally finds that secretory action is less marked than in the other cases, and it is liable to be localized to various parts of the surface.

Both teased or cover-glass preparations and sections of stimulated skins have been studied.

(a.) Teased Preparations of Stimulated Skins.

These have been prepared from chloroform vapour stimulated animals. As one would expect, such preparations differ from the normal mainly in this, that very few normal club cells with closed vesicle are to be found ; on the other hand, the field teems with "fibre masses" in various stages of disintegration into slime fibres. Such forms as are figured in figs. 4a and b, Plate 30, are the rule.

At the same time the number of extruded nuclei is generally very great.

The "escape cells," so abundant in the teased preparations of the normal winter Eels, are conspicuous by their absence in similar preparations from stimulated skins. The process of development of the fibre mass from the club cell and the setting free of the contents of the vesicle is far more energetic than in the normal slow secretory process, and instead of a slow "shelling out" of the vesicle and its contents, an almost explosive disruption of the structure appears to occur in stimulation by chloroform vapour.

One must, however, turn to the sections in order to see how energetic this secretory action may be.

(b.) Sections of Stimulated Skins.

(1.) Chloroform Vapour Method.

As above mentioned, the epidermis of a chloroform vapour stimulated Eel becomes loosened in texture. This loosening is dependent upon three changes :—(α) The eruptive production of spiral fibre masses from the club cells within the epidermis ; (β) the production and swelling of goblet cells ; (γ) the passage of numbers of fibroblasts into the epidermis.

(α.) *The Changes in the Club Cells upon Stimulation.*—A section through a chloroform vapour stimulated skin at once reveals the fact that few, if any, of the club

cells remain in their normal condition. Curling fibre masses (fig. 36, Plate 32, and Photo C, Plate 33), with granular matter round their upper ends, have now taken the place of club cells with closed vesicles. There can be no doubt that the process of production of the spiral fibre masses from the normal club cells has been the result of the excitation, and instead of the process occurring only in the more developed clubs nearer the surface, it has occurred in the lower layers to almost equal extent. If the excitation has not been severe the surface epidermis may still remain intact (fig. 36, Plate 32, and Photo C, Plate 33), but should it be intense, this eruptive uncoiling of fibre masses may lift the surface cells, with a general disintegration of the epidermis as a resultant. A magnificent example of this will be found in Photo B, Plate 33, which should be contrasted with that of a normal skin in the lowest phase of secretory activity presented in Photo A of the same plate.

The question at once arises : Is this action of chloroform vapour a direct one upon the club cells? or, Is it a reflex action demanding nervous ties with the cord?

The answer is certainly to be given in favour of the latter query, for the following reason, that *exposure of exsected skin to the action of chloroform vapour does not produce the effect.*

There is never any evidence of " an eruption " when the vapour is allowed to act upon the removed skin. Fig. 35, Plate 32, is from a piece of removed skin subjected to chloroform vapour for about fifteen minutes, and is to be contrasted with fig. 36 of the same plate, where the animal with intact spinal cord was stimulated by the vapour. There is, then, physiological evidence of nervous connections for the club cells, though I have not as yet been able to convince myself of the fact anatomically.

It should here be noted that in former experiments upon the electromotive properties of the skin of the Eel (34) I found that exposure of exsected skin to the action of chloroform vapour caused a fall of potential, and I believe that the direct action upon the protoplasm of the secretory elements is narcotic, though in early stages one may have a reflex excitatory effect in the animal with intact cord.

In this reflex stimulation of the club cells it is apparently only the formed cells that react to the excitation; at any rate I have seen no evidence of chloroform excitation leading to a rapid production of new individuals from the palisade cells. Yet it is difficult to imagine how any nerve connection with the club cells can be retained after they have been set free from the palisade layer. Possibly some conducting protoplasmic connection, that has escaped me, is retained between the palisade cells and the club cells, the nerve connection being solely with the former.*

(β.) *The Production of Goblet Cells.*—At the same time that this extraordinary change is produced in the club cells, a great production of goblet cells also appears to take place upon stimulation with chloroform vapour. Normally a small rounded goblet cell occurs here and there above the apices of the palisade cells. After stimulation a

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* Or it may be that we have to deal only with *contiguity* of nerve fibres to irritable structures.

dense crop is usually found above the palisade layer (fig. 36, Plate 32), generally giving evidence of being subjected to pressure by their elongated form.

At the same time the older goblets in the upper layers became swollen, and even in osmic and Flemming hardened material have lost their granularity, which is replaced by a network.

This change is apparently due to a local action of the vapour, for I have not generally found much difference in number of goblet cells between the exsected skins and those stimulated on the animal. The paucity of goblets in fig. 35, Plate 32, is rather exceptional.

(γ.) *The Passage of Fibroblasts into the Epidermis.*—The quantity of fibroblasts in the lower layers of stimulated skins is one of their most marked features. This is clear in fig. 36, Plate 32.

This change can, of course, only occur so long as the skin is on the animal, and, consequently, exsected skins subjected to chloroform vapour do not show this peculiarity.

That these cells are really exuded leucocytes is forced upon one when one observes transverse sections of blood-vessels. Capillaries lie some $5\,\mu$ or $10\,\mu$ below the basement membrane, and in stimulated skins it is easy to trace the leucocytes from the vessels towards the epidermis. The extent to which diapedesis may occur is depicted in fig. 34, Plate 32, from a section across the pectoral fin.

In the rapid processes of reflex secretion evoked by chloroform vapour, it would appear that all these extruded cells are not converted into connective tissue corpuscles, for one finds them unaltered in the upper layers of the epidermis, and sometimes large masses of small cells, $5\,\mu$ to $6\,\mu$ in diameter, are found lying upon the surface of the stimulated skin. A good instance is seen in Photo B, Plate 33.

It is, of course, possible that many of these nuclei are from extruded club cell vesicles, but since I find a distinct cell body round most, I am inclined to consider them as undeveloped fibroblasts.

(2.) *Stimulation by Faradization.*

Faradization does not appear to act as such a strong stimulus as chloroform vapour, but, since it can have no narcotic after-effect, can be used for observing the effects of a long period of moderate excitation, in contrast to the violent but brief reflex action of chloroform. Prolonged faradization supplies skins without a trace of surface epidermic cells, though one finds that multiplication in the lower layers is very active. Again, young club cells are frequent in the lower layers, but are usually directly extruded as such, vesicle and all, so that in Eels faradized in air, a collection of extruded club cells is found upon the surface, many giving signs of subsequent disintegration. An illustration of this is seen in Photo D, Plate 33. Fœttinger (8) has already figured these extruded club cells in *Petromyzon* (see figs. 10, 11, and 12, Plate 31, of his paper). The nuclei of the palisade cells also exhibit signs of division to a very marked extent.

No marked production of goblet cells, on the other hand, is evident in faradized skins. Fibroblasts are, however, very numerous in the lower layers (Photo D, Plate 33), and extravasated red corpuscles are often found among the fibres of the corium.

(3.) *Mechanical Stimulation.*

The changes met with in the skins of Eels that have writhed in the process of capture are, as would be expected, less marked and often localized.

They have already been incidentally referred to in previous pages, since such moderate conditions of stimulation could scarcely be separated from the account of the slow process of normal secretion. Figures in illustration are given in Plate 30, fig. 5, and Plate 33, fig. 29.

The most marked effect, therefore, of subjecting Eels to artificial stimulation is to cause a rapid production of fibre masses from the club cells. In stimulation by chloroform vapour, this process is almost "volcanic" in its energy, the epidermis being so loosened that slight pressure is sufficient to cause removal of the upper layers. The animal, therefore, actually leaves a considerable part of its epidermis behind when it is gripped, the regenerative palisade layer, however, always remaining. The increase of fibroblasts, which also occurs, is suggestive of the idea that these may act as a temporary protection and support by development into connective tissue corpuscles, during subsequent regeneration of lost elements.

NOTE UPON THE ACTION OF ATROPINE.

Eels are very resistant to intoxication with atropine, and live for days in fairly strong solutions. It is, however, possible to obtain histological effects, which are of some interest and are indicative of a paralytic action of the drug as regards secretion.

The skins of atropinized Eels teem with fibre masses and escape masses, yet there is no evidence of rapid removal of the surface or escape of the secretory elements or their products. Figures from sections of an atropinized Eel, which refused to give any excitatory electrical variation upon faradic excitation (see my former paper, 'Phil. Trans.', vol. 184, B, p. 360), are given in Plate 32, figs. 31a and b. It would appear that the appearances there seen are to be accounted for in the following manner. At first, the introduction of the animal into a foreign medium has induced a secretory action, but this has soon become paralyzed, so that the fibre masses, &c., first formed are retained in the epidermis. The whole epidermis of such Eels is, too, actually on an average thicker than normal, while in Eels stimulated for long periods (faradization or life in pilocarpine bath), it becomes thinned.

The club cells have not the normal appearance (fig. 31b), the material of the head having undergone change into a granular material instead of the ordinary more clearly defined fibre mass material. The club in this figure has developed to the full, yet it has never left the palisade layer. Fibroblasts too, are exceedingly rare, while

on the other hand, ordinary epidermic cells are found compactly set right down to the palisade cells.

The skins certainly have the appearance of having been stimulated to secrete rapidly, but the process subsequently paralyzed.

CONCLUSION.

It is evident from the foregoing account that the secretory process in the skin of the Eel presents several points of peculiarity and interest. The demonstration of a secretion of threads from the club cells points to a similarity of function of these cells in such otherwise widely separate Fish as the Eel and *Myxine*. The process is certainly better developed in the latter animal, and it has, moreover, the advantage of special epidermic involutions devoted to manufacture and store of threads ; yet in both, the whole club cell is cast off and mainly in the form of threads.

The fact that *Petromyzon* produces a thread secretion was to be expected, but had not been before clearly demonstrated, though the extrusion of the club cells was known.

What relation, if any, these thread-producing epidermic cells in Fish have to those of larval Amphibia described by LEYDIG (10) and EBERTH (11), it is not my place to speculate upon. As regards the function of these threads in the slime of the Eel, I have already stated my opinion that they are of value in causing the slime to adhere to objects with which it is brought in contact. In *Myxine*, however, I cannot but think that the cloud of thread-penetrated slime that the animal is capable of discharging, must be of service in aiding it to attack the far more powerful Fish that often become its host.

The physiological process involved in the sudden development of a spiral fibre mass upon stimulation, leading to a veritable upheaval of the epidermis, presents a problem in cell mechanics, at present inscrutable, but of great interest, though the effect in giving the animal the power to loosen the surface of its skin explains the proverbial slipperiness.

The connective-tissue network of the epidermis appears as a special arrangement for consolidating an otherwise extremely labile structure, and the observation that during excitation provision is made for a fresh supply of supporting cells by an inroad of fibroblasts would almost be expected from the nature of the case.

In fine, the following conclusions may be summarized :—

1. The secreting elements of the epidermis of the common Eel consist of goblet cells and club cells, both direct descendants of the cells of the palisade layer. The former supply a mucin, the latter threads and a material appearing as fine granules in the slime.

2. The goblet cells contain mucin granules, and, after reaching the surface and discharging their load, are capable of undergoing regeneration, by growth of the protoplasmic foot and re-formation of mucin.

3. The threads of the slime resemble those of *Myxine glutinosa,* but are usually of finer texture. As in *Myxine,* they are developed from the club cells, but there are no special glandular involutions of the epidermis. The club cells of *Petromyzon fluviatilis* also supply slime threads.

4. The granular material of the slime is the contents of vesicular spaces developed in the club cells in the immediate neighbourhood of their nuclei, and is set free enclosed in a lattice work developed by vacuolation of the surrounding material and finally extruded, carrying with it the original nucleus of the club cell.

5. The remainder of the club cell after extrusion of its vesicle and nucleus becomes a spirally coiled fibre, which finally breaks up into the fine fibrils of the slime.

6. Severe stimulation, especially by the vapour of chloroform, applied to the intact animal, causes so sudden a development of the coiled fibres from the club cells that the surface of the epidermis is thrown off and the secretory products set free *en masse.* This process is of reflex nature, for similar excitation applied to excised skin is without effect.

7. A system of connective tissue cells, distinct from chromatophores, exists in the epidermis developed from cells which are direct descendants of leucocytes, and which can be traced from the bloodvessels of the corium through the basement membrane of the epidermis. The number of these wandering cells in the epidermis is greatly increased by stimulation, probably with a view to providing subsequent support to the secretory elements during regeneration.

APPENDIX.

Histological Procedure.

Isolation of Elements.—The difficulty of isolation of the fresh elements of the epidermis, on account of the viscous nature of the structure, compelled me to resort to maceration. RANVIER's "third part" alcohol was found to deform the elements less than any other dissociating liquid, and was used throughout.

The skin was placed for 24 to 48 hours in this fluid, and then either shaken in water to separate the elements, or cover-glass preparations of the softened epidermis were made in the usual manner. In preparing the elements of the slime the cover-glass method was used, except in cases where the flocks of secreted matter rising from an Eel in a bath were collected, in which cases the material was placed at once in $\frac{1}{10}$ per cent. osmic acid solution, and, after a few days, teased in dilute glycerine. The dry cover-glass preparations were either stained directly with picro-carmine, Biondi stain, methylene blue, or soluble blue, or, if to be stained with thionin, first treated with corrosive sublimate. The macerated material not made into cover-glass preparations was either stained by irrigation in small teased masses on the slide, or stained in bulk, and subsequently teased in dilute glycerine. The action of digestive ferments was watched in the hanging drop in an ENGELMANN's gas chamber placed upon a SCHÄFER's warm stage kept at 37° C.

Preparation of Material for Sections.

The following fixing reagents have been used from time to time. Osmic vapour. FLEMMING's fluid. PERENYI's fluid. Picric acid (saturated aqueous solution). Corrosive sublimate (saturated aqueous solution). Nitric acid, 10 per cent., for 24 hours, followed by osmic acid, 1 per cent., for 24 hours. Equal parts of saturated aqueous corrosive sublimate solution, and 1 per cent. chromic acid solution. Absolute alcohol containing 25 per cent. of glacial acetic acid. In all cases a graduated series of alcohols was subsequently employed.

The best results were obtained with FLEMMING's fluid and the nitric-osmic method, though the corrosive sublimate preparations were very reliable.

Staining.—This was usually effected in bulk. A good nuclear saffranine from GRÜBLER used in semi-alcoholic solution gave the finest results upon the material fixed in FLEMMING's fluid and by nitric and osmic acids. Methylene blue was also valuable. The corrosive-hardened material was stained in bulk usually with Biondi stain or hæmatoxylin.

If staining was effected after cutting sections, saffranine, hæmatoxylin, and soluble blue were used for the Flemming fixed material, or thionin after previous treatment of the sections with corrosive sublimate solution. I found the mucin reaction with thionin succeeded as well in the Flemming fixed material as in corrosive fixed epidermis, provided the former was first treated with corrosive sublimate, but, unfortunately, the specimens soon fade. Sections of material fixed with corrosive sublimate were stained with thionin, picro-carmine, Biondi stain, or hæmatoxylin.

In all cases where it was necessary to hold parts together, or where the preservation of the condition of the surface was absolutely necessary (see, for instance, Photos B and D, Plate 33), the sections were cemented to the slide by floating on in 50 per cent. alcohol while still in paraffin, and subsequent drying at 35° C.

The paraffin method, with xylol, has been used throughout for obtaining the sections.

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DESCRIPTION OF PLATES 30-33.

ZEISS' apochromatic objectives and oculars have been used throughout, and the drawings have, without exception, been executed with the Abbe camera lucida. The photographs in Plate 33 were taken with the same system of objectives supplied with projection oculars, the source of light being a 3000-candle power arc lamp. My thanks are due to Professor CLAXTON FIDLER, of the Engineering Department of University College, Dundee, for his kindness in supplying me with current for the lamp.

PLATE 30.

Fig. 1. × 600. From a "cover-glass preparation" of the slime of a "normal" Eel, stained with picro-carmine. The characteristic yellow reaction of the threads of the slime is shown, also relative size of extruded nuclei and granules. An extruded goblet cell is present on the left.

Fig. 2. × 600.   Origin of club cells from palisade cells.   FLEMMING's fluid and
saffranine ; *a*, *b*, *c*, and *f* show the method of origin by lateral division
of the palisade cell at right angles to its longer axis.   The darkening of
the apex of the young club cell by the FLEMMING's fluid is visible in all
cases.

Fig. 3. × 600.   Stage in development of club cells previous to the formation of the
vesicle.   Fixing and staining as in fig. 2.   Note the granular matter
densely stained with the saffranine, in the region of the nucleus.

Fig. 4*a* and *b*. × 600.   Two stages in the development of the slime fibres from the
" fibre-masses" of the club cells.   From a " cover-glass" preparation of an
epidermis stimulated by chloroform vapour.   RANVIER's alcohol and picro-
carmine.   The same yellow reaction is evident as in the formed fibres of
the slime (fig. 1) in both cases, and in *b* the breaking up into the finest
fibres is seen.

Fig. 5. × 600.   Developed "fibre-masses" in process of extrusion.   From a winter Eel
that had struggled during killing.   FLEMMING's fluid and saffranine.   Note
the dark coloration of the fully-formed " fibre-mass," and compare with
that of the apices of the club cells still possessing a vesicle.   Also, the
absence of saffranine staining of the contents of the club vesicle should
be contrasted with the marked coloration of the contents of the goblet
cells.   Note also a considerable number of fibroblasts in the lower layers,
many of which exhibit mitotic figures.

Fig. 6*a*, *b*, and *c*. × 600.   Similarity in staining reaction of the contents of the club
vesicle at early and later stages with that of the " escape mass."   Winter
Eel, FLEMMING's fluid and soluble blue.   The contents of the fully
developed vesicle and " escape mass " are distinctly granular, that of the
young vesicle is homogeneous.

Fig. 7. × 600.   Division of palisade cells.   FLEMMING's fluid and saffranine.   The
upper examples are from sections parallel to the surface of the skin, the
lower are cut at right angles to the surface.   In the upper note the
lateral bulging, staining yellowish-brown, and indicative of the commence-
ment of differentiation of the club cell body material.   In the lower, the
deeper staining of the apices of the conical palisade cells is noticeable.
No mitotic figures are to be seen.

Fig. 8. × 600.   Medium stage in the development of a club cell and a goblet cell.
FLEMMING's fluid and saffranine.   In the vesicle of the club cell, note a
curved line, separating a denser lower part from a less dense upper part.
This line passes upwards, as development proceeds, and probably marks
the edge of the membrane of the " escape cell," which is becoming differen-
tiated from the wall of the club.   Note the "foot" and marked nucleolus
of the goblet cell, its granular contents, and difference in staining reaction

to those of the club vesicle. The dividing palisade cell immediately below the goblet cell is giving rise to an ordinary epidermic cell.

Fig. 9*a* and *b*. × 600. Origin of goblet cells from palisade cells. FLEMMING's fluid and saffranine.

9*a*. The transformation of some of the protoplasm of the young goblet cell into a saffranine staining mucigen, even before the new cell has parted from its mother, is seen in 1, 2, and 4, and should be contrasted with the early stages and staining reactions of the young club cells in fig. 2. 2 is a case of origin by lateral division; 1 and 4, by transverse division of the palisade cell, though in the latter case the two nuclei of the palisade cell show that a lateral division will take place shortly. 3 shows how early the characteristic shape is attained in many cases.

9*b*. 6 shows that the characteristic shape may be developed even before separation from the parent, and also (as, too, in 8) the great length to which the outwardly directed process of the palisade cell may reach. In 7, a goblet cell is being thrust upwards by an ordinary epidermic cell with characteristic elongated nucleus.

Fig. 10. × 600. Regenerating goblet cell (re-formation of mucigen). FLEMMING's fluid. Thionin, after treatment with corrosive sublimate. Winter Eel. Note the characteristic mucin reaction with thionin in the protoplasm of this cell, and contrast with the ordinary blue colour of thionin taken by the nucleus of this cell and those of the surrounding epidermic cells. A wandering pigment cell is also to be seen.

Fig. 11. × 600. Goblet cells in earlier stages of regeneration than that in fig. 10. Preparation as in case of fig. 10. The cell on the left consists of the protoplasmic "foot" alone, containing granules staining black with osmic acid. The theca, with its load of mucigen, has disappeared, but no new supply has been yet formed, as in fig. 10. The two cells on the right indicate stages in the growth of the protoplasm of the "foot," in cases where the first load of mucigen has not been completely discharged. The darkened granules are again seen here; they are never seen in the "foot" of ripe cells (*cf.* fig. 12*a*). The nucleolus is, as a rule, absent in the regenerating cells.

Fig. 12. × 600. Ripe, discharging, and regenerating (earliest stages) goblet cells. FLEMMING's fluid. *a* is stained with methylene blue; *b*, *c*, and *d* with hæmatoxylin. In *b* and *c*, which are discharging, the stoma of the theca and the projecting "pfropf" of mucus are seen. *a* is a cell ripe for discharge, the theca still being intact. *d* is an intermediate stage in regeneration between the cells on the extreme right and left of fig. 11.

Fig. 13. × 435. Section of winter Eel killed "instantaneously," and in lowest phase of secretory activity. FLEMMING's fluid. Thionin, after treatment with

corrosive sublimate. Note the reddish-violet reaction of the contents of the goblet cells, and compare with the absence of staining of the contents of the club vesicles and the blue colour of the nuclei throughout. A "fibre mass," tending to be spiral, is seen on the left, and a point of escape, lined by a few fibrils, is seen above. A thick layer (7 or 8 cells) of superficial epidermic cells is present.

PLATE 31.

Fig. 14. × 600. Convoluted fibre, discharged by an Eel placed in a bath of pilocarpine solution. $\frac{1}{10}$-per cent. osmic. Dilute glycerine.

Fig. 15a and b. × 600. Club cells with dividing nuclei. Winter Eel. RANVIER'S "third part" alcohol. Carmine. Dilute glycerine. Note the lattice work in the wall of the vesicle and the "prickles" upon the outer surface of the body of the cell.

Fig. 16a and b. × 600. Club cells with two vesicles, each holding a nucleus. Preparation as in fig. 15. In a, note the granular core passing down the stalk from the vesicle.

Fig. 17a, b, and c. Extrusion of the "escape cell" from the club. a and c, × 435. b, × 600. Preparation as in fig. 15. The lattice work in the wall of the "escape cell" is especially clear in b. In c, the presence of a second nucleus indicates the possibility of formation of a second "escape cell."

Fig. 18a and b. Development of a coiled "fibre mass" without the formation of a definite "escape cell." (The remainder of the vesicle is in such cases referred to as an "escape mass" in the text.) a, × 435. b, × 600. Preparation as in fig. 15.

Fig. 19a and b. Secondary vacuolation of "fibre mass." a, × 435. b, × 600. Preparation as in fig. 15. Note granularity in secondary vacuolation spaces in a, similar to that occurring in the stalk in figs. 15, 16, and 17.

Fig. 20a, b, c, and d. × 600. Stages in the vacuolation of the club body material, leading up to the freeing of the "fibre mass." Preparation as in fig. 15. The vacuolation and formation of a lattice work, filled with granular matter, is seen commencing in a, and further developed in b. In c, this granular material has become freed in the process of maceration, so that the trellis of the residual unaltered club head material is very distinct, In d, the "fibre mass" is free. The "prickles" noticeable in figs. 15, 16, 17, and 18 are retained upon the lower end of the developed "fibre mass."

Fig 21a and b. × 600. Large isolated "escape cells." Preparation as in fig. 15. In this form, the greater part of the club body material is converted into granular material, and the "fibre mass" remains attached as a convoluted coarse filament.

Fig. 22a, b, and c. × 600. Isolated "escape cells." Preparation as in fig. 15. In a
some of the trellis produced in the vacuolation of the club head is still
adherent; b has no thick wall of club head material, and is an interme-
diate form between an "escape cell" and "escape mass." In c the thick
wall is complete only on one side.

Fig. 23a, b, c, d, e, f, and g. × 600. Preparation as in fig. 15. These figures show the
great variation in size and structure of "escape cells" met with in teased
preparations; a and b show the possibility of division of the nucleus;
b, c, and d show the nucleus in process of extrusion; e and g show how
extremely small these cells may be in comparison with such forms as
fig. 21a and b; f (as also in fig. 22a) shows a stout strand of club body
material that has been ruptured in the process of "shelling out" of the
cell. In all, the outer surface is seen to be beset with spines of the
original lattice work—a fact also frequently clear in sections c, f, fig. 6c,
Plate 30.

N.B.—All the figures upon this Plate, with the single exception of
fig. 14, are from one winter Eel, killed "instantaneously."

PLATE 32.

Fig. 24. × 600. Pigment cells of the epidermis, from a section hardened in FLEM-
MING'S fluid.

Fig. 25. × 600. Fibroblasts, isolated by RANVIER'S alcohol. Carmine. Glycerine.
In passing from left to right, all stages, from the simple lymphocyte-like
cell to the small connective tissue cell with long fine processes, are to be
observed.

Fig. 26. × 600. Ordinary epidermic cells, from various levels. Preparation as in
fig. 25. a, b, c, and l, with elongated nuclei, are from the lower layers;
d and e, from a higher level; h and i, from near the surface; f, g, j, and
k are dividing cells from near the surface, but lack mitotic figures in
their nuclei. It will be noted that the more superficial cells present
"prickles."

Fig. 27. × 600. Isolated goblet cell. Preparation as in fig. 25. The plicated wall
of the empty theca and the marked nucleolus of the nucleus are to be
noted.

Fig. 28. × 600. Formation of the vesicle of the club cells. From sections of mate-
rial hardened in FLEMMING'S fluid, and stained with saffranine. This
condition is presented in the stage following that depicted in fig. 3,
Plate 30. The intimate relation of the vesicle to the nucleus is to be noted,
as also the granular transformation of the club body material which pre-
cedes the enlargement of the vesicle. The vesicle may start on the outer

side of the nucleus a, f, g, and i, with granularity only at the opposite pole, or *vice versâ*, h and k ; on the other hand, it is possible for it to arise simultaneously upon both sides, b, c, d, and e.

Fig. 29. × 435. An escaping " fibre mass," lifting surface cells. From a winter Eel that had struggled during capture. FLEMMING's fluid and saffranine.

Fig. 30. × 435. An " escape cell " and related " fibre mass " *in situ*, in a section. Same Eel and preparation as fig. 29.

Fig. 31a and b. a, × 160. b, × 600. Appearances in the epidermis of an Eel that had lived two days in a solution of atropine. FLEMMING's fluid and saffranine. In a, " fibre masses " and " escape masses " are seen to exist at all levels of the epidermis, and a peculiar granularity is noticeable round about the upper ends of the " fibre masses." The general appearance of the section is suggestive of an initial secretory activity, which has been sub-sequently brought to a close by the paralyzing action of the drug. b shows the appearance of a club cell still in contact with the palisade layer, which, though developed to the full, has never left its position of origin. A developed fibroblast with long processes is seen ensheathing this club, at the upper part of the figure.

Fig. 32. × 435. Section of outer edge of lip. Picric acid and hæmatoxylin. Note the absence of club cells, presence of a goblet cell, and one of the well-known sense organs.

Fig. 33. × 600. Point of escape of a " fibre mass." From a winter Eel. FLEMMING's fluid and thionin.

Fig. 34. × 600. Diapedesis. From central vessels of pectoral fin. Picric acid and hæmatoxylin. These extravasated cells are quite indistinguishable from the young fibroblasts found in the lower layers of the epidermis.

Figs. 35 and 36. × 435. Fig. 35 is a section of skin subjected to the action of chloroform vapour *after removal*. Fig. 36 is from the skin of an Eel simply decapitated and then exposed to the vapour. Both were fixed with corrosive sublimate and stained with hæmatoxylin. In fig. 35, most of the club cells are in the earlier stage, with closed vesicle. In fig. 36, only curled " fibre masses " are found, with some granular débris around them. There is also to be noted, in fig. 36, a relative increase in the quantity of goblet cells and fibroblasts in the lower layers. *Cf.* also Photos B and C, Plate 33.

PLATE 33.

The prints on this Plate are Collotypes, direct from untouched negatives.

Photo A. × 320. Epidermis of a " normal " winter Eel. FLEMMING's fluid and saffranine. Note below the club cells, with closed vesicles, and the

conical palisade cells; above, the free " escape masses." This print represents the appearances of the lowest phase of secretory action.

Photo B. × 175. Later stages of stimulation by chloroform vapour. Nitric and osmic acid. Saffranine. Note the " eruption" of fibre masses (darkly stained) causing considerable disruption of the layers of the epidermis. Above is seen a compact mass of small cells (5μ), which are, probably, extruded fibroblasts. A pad of coagulated mucus, containing a few epidermic cells, separates this mass of small cells from the parts below. This print should be contrasted with the appearance of the resting skin seen in Photo A.

Photo C. × 300. Early stages of stimulation by chloroform vapour. Compare with Photo B. Nitric and osmic acids. Saffranine. A good example of a coiled fibre mass is seen on the right.

Photo D. × 175. Epidermis of a summer Eel faradised for an hour. FLEMMING'S fluid and saffranine. The superficial epidermic cells are absent, and the surface consists of a mass of extruded fibre masses and club cells. An escaping club cell is evident upon the left. Note the large number of fibroblasts in the lower layers.

Photo E. × 900. Passage of fibroblasts from the corium into the epidermis. Picric acid and hæmatoxylin. Fibroblasts can be seen upon the corium side of the basement membrane, one is fixed as it passes through, another between two palisade cells, and others lie free in the epidermis. In two of these latter a protoplasmic process is evident upon one side of the cell. Note the relative sizes of the nuclei of the palisade. cells and the fibroblasts.

Photo F. ×450. Connective tissue network in the epidermis. Section parallel to the surface of the skin about midway between surface and basement membrane. Picric acid and saffranine. The bodies of the club cells have shrunk in preparation,.and so allow the branching cells to he easily visible.
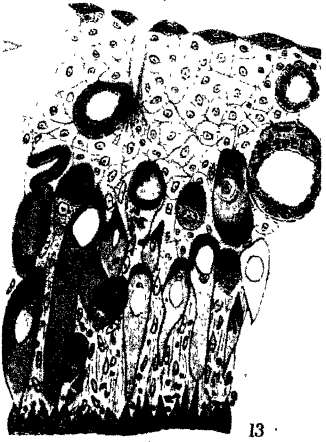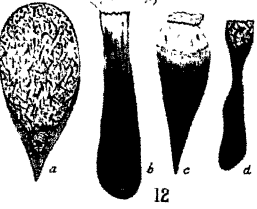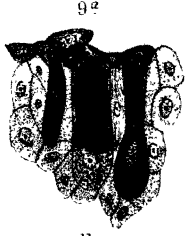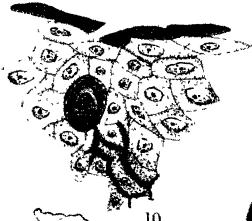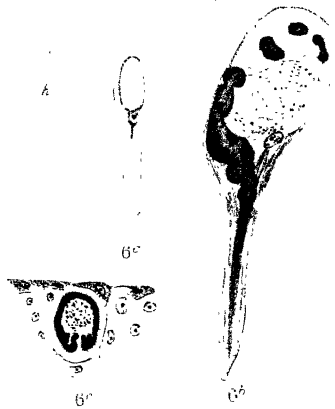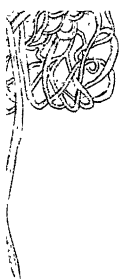
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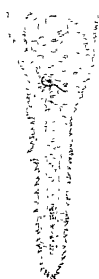
15^b

16^a

16^b

17^a

17^b

18^d

18^b

19^a

19^b

20^b

21^a

21^b

20^a

a.

b

22

c

24   25   26
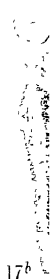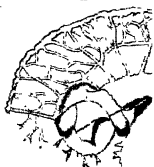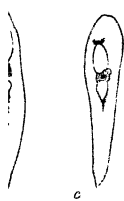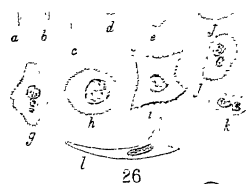
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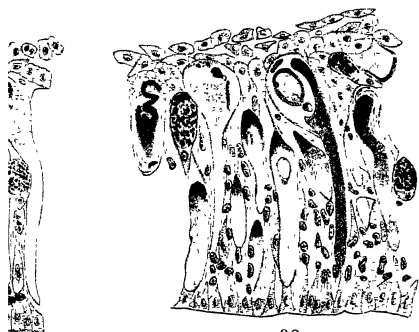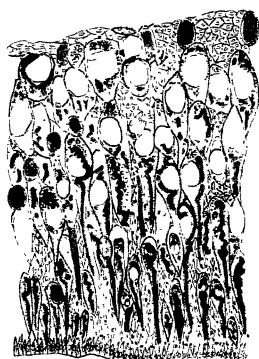
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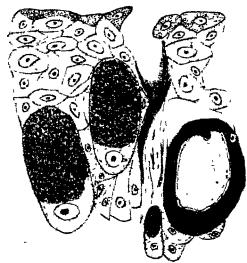
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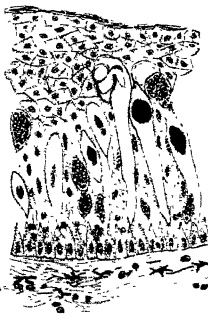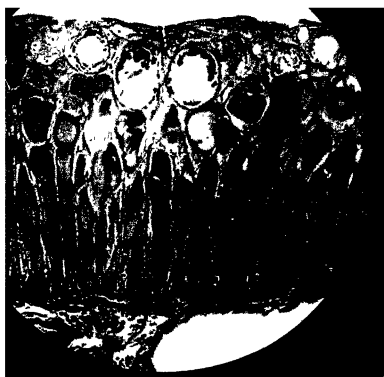
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D
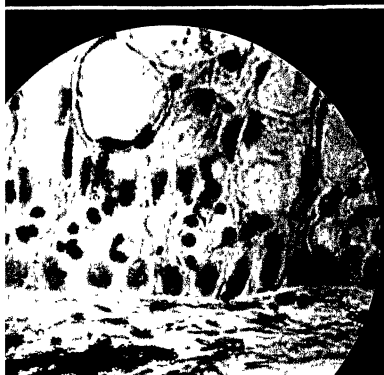
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IX. *On the Constitution and Mode of Formation of "Food Vacuoles" in Infusoria, as illustrated by the History of the Processes of Digestion in* Carchesium polypinum.

By M. Greenwood, *Lecturer of Girton and Newnham Colleges, Cambridge.*

Communicated by J. N. Langley, *F.R.S.*

[Plate 34.]

In the course of some unpublished experiments on the rôle of acid in protozoan digestion, I had occasion to use coagulated white of egg as a food for certain colonial Vorticellidæ. This substance, diluted with water before coagulation, and therefore given to the animals as a finely granular precipitate, is usually found after enclosure in the form of smooth oval or spherical masses (Plate 34, fig. 2, *C.s.*), which are widely different both in refractive characters and in size, from the minute irregular particles offered for ingestion. So striking is the contrast, and so constant is its occurrence with this form of food, that I attempted to watch the process by which the spherical ingesta are shaped : in this attempt I was struck by the clearness with which some characteristic features of the process are demonstrable in *Carchesium*, and realized that the very act, with the performance of which I was especially concerned, was apparently bound up with the operation of a mechanism undescribed in existing descriptions of digestion in the Protozoa. This led me to record, in the paper which follows, the result of sequent observation of the phenomena of ingestion, digestion, and ejection in *Carchesium*; for, while the intracellular solution of food is as truly *without* the cell substance as in the case of *Amœba*; while, indeed, there is no fundamental fact established for the digestion of that animal which does not find a parallel here, —the digestive cycle is shortened, unfamiliar details of the process are striking, and even the more familiar events occur with a dramatic vividness which makes them almost strange.

The animals I watched (*Carchesium* and *Epistylis*) were kept in hanging drops of water, and were thus under no unusual pressure. Various solutions and suspended

particles were added at different times to the water of these hanging drops, and, in order to examine the results of the addition of each substance, I used, as a rule, Oc. 3, Obj. F. (ZEISS) or Oc. 3, Obj. $\frac{1}{12}$ oil immersion (ZEISS or LEITZ), working sometimes by daylight, sometimes by artificial light. The focal length of these objectives does not allow the examination of animals in any great depth of encircling fluid; but such signs of lesion as extreme convexity of the peristome, the development of an enormous contractile vacuole, definition of the outline of the nucleus, are so prompt in their appearance in these Vorticellidæ, and so unmistakable, that it is possible, by the use of recurrent immersions in abundant water, to watch the same colony for many successive days.

Carchesium polypinum grows in pedicellate clusters of varying size; the disadvantage which (from an observer's point of view) attends the marked contractility of the stalks of a healthy colony is compensated by the comparatively large size of the polypes, and the transparency of their cell substance. Various species of *Epistylis,* more manageable because they are mounted upon relatively rigid pedicels, are often too opaque for accurate study.

Any interest which the food of *Carchesium* may have, centres almost exclusively round the events which mark its actual progress through the cell substance of the animal; it is hardly to the point, then, to discuss here the varying and somewhat fanciful terminology which former writers have applied to the funnel-shaped oral tube which collects and guides matter before its ingestion. It seems needful to say, however, that two points have formed the subject of much discussion; the first is the position of the mouth, the second is the internal continuation of the oral tube. In fig. 9 I have duplicated the diagrammatic representation of the isolated "digestive canal" in *Epistylis flavicans* given by GREEF,[*] in order to bring out, by corresponding letters, the differences in nomenclature which part him from some preceding writers. According to GREEF, the external opening of this canal may be termed the *mouth;* a slightly sinuous *pharynx* follows, spacious at first but narrowing considerably in its course, and a small dilated sac, the *œsophagus,* ends this internally. Since waste matters are ejected constantly from a ridge which lies between the middle and outer thirds of this pharyngeal tube, it is clear that the entrance of food and the exit of débris take place for a certain distance through a common passage. It may be on this ground that some writers, notably CLAPARÈDE and LACHMANN,[†] place the mouth internally of the anal ridge (*cf.* fig. 9), and give the name of *vestibule* to the outer one-third of the *pharynx* of GREEF. The tube as it narrows internally is, according to them, the *œsophagus,* and the terminal dilated sac the pharynx. In this paper I adopt the terminology of GREEF, which at least follows the sequence of regions in a true alimentary canal; his theory that a collapsible tube curves for some distance from the œsophagus into the substance of the animal, I cannot support,

* R. GREEF, Archiv f. Naturgesch. (WIEGMANN), vol. 37, 1871.
† CLAPARÈDE ET LACHMANN. Études sur les Infusoires.

from observations made on *Carchesium*. It may be possible that there is structural difference between the genera (I have said that the statements of GREEF refer to *Epistylis flavicans*), for different specimens of *Carchesium* do seem to show slight variations in the extent of the pharynx; but the existence of anything like a permanent, far-reaching tube is disproved, I think, by evidence which can be brought forward more suitably later. As a rule, the pharyngeal tube (fig. 5) placed somewhat obliquely, ends about midway to the basal attachment of the polype; it is ciliated throughout and separated from the oval œsophagus by a slight annular constriction. Into the œsophageal sac, all ingesta are whipped by ciliary action, and they start from its most internal point on their intracellular career as constituents of the vacuole of ingestion. It is this career which I propose to trace, grouping its minor details in sequence about such as are more important, and postponing any discussion of their significance until the tale is told. And it may be well to preface the whole description with a brief account of those events in the gullet which are immediately antecedent to ingestion.

EXTRACELLULAR PHENOMENA.

The ingestion of particles by *Carchesium* is in a certain sense selective, but the selection depends in no way on the nature of the particles, but only on their size. According to GREEF,[*] the stream of solid granules which may enter the mouth of *Epistylis flavicans* is so directed by two delicate valvular membranes that some of its constituents gain the internal parts of the pharyngeal tube, while some are discharged at once to the exterior. These membranes are figured as occurring at that knee-like bend which characterizes the pharynx in *Epistylis*, and it is quite possible that in *Carchesium*, where the immediate rejection of most particles primarily swept into the mouth is clear enough sometimes, the directing action becomes almost that of a strainer. I have followed the ingestion of a compact solid, measuring 6μ by 3μ, but any fragment of enclosed matter which is larger than this, exceeds it in one diameter only, or is one of such loose flocculi as make up a freshly fallen precipitate of alizarin sulphate. Among ingesta which are linear and relatively long, we must count some bacterial filaments and the acicular crystals which alizarin sulphate forms with lime in water. In the great majority of cases, when the polype deals with these ingesta, short rods gather in the gullet and help to form the vacuole of ingestion; I think it reasonable to suppose, therefore, that the oral and pharyngeal cilia have some power of breaking up these slender threads, in addition to the selective action by virtue of which they hinder the entrance of particles which exceed a certain size. In some rare cases one can watch the enclosure of a bacterial filament (equal in length, perhaps, to the polype which encloses it) without any preliminary fragmentation. The act is carried out by the protoplasm of the animal, independently, it may be, of

[*] R. GREEF, *loc. cit.*

any immediate secretion of fluid; and the end of the bacterium first enclosed is coiled upon itself, sometimes even while the free posterior end, which helps eventually to thicken the coil, lies freely in the pharynx. The process is relatively slow, and occurs so rarely that there is some temptation to regard it as a distortion of normal ingestion; there seems little room for doubt, at least, that the most acceptable particles are very minute,—Indian ink suspended in water, the smaller fatty globules of milk, carmine, also finely divided, and the coagulation precipitate from diluted white of egg to which I have referred above.

While, however, it is clear, from these facts, that the ingesta of *Carchesium* are made up of particles which vary considerably in size, the connection between this variation and the fashion of their preliminary accumulation in the gullet is less evident. The lowest pharyngeal cilia, acting downwards from the annulus which I have described, beat the solid matter within their reach into the most internal extremity and then into the body of the œsophageal sac (which may dilate considerably, fig. 5, *ing.*). But the admixture of water, or rather the medium in which the animal is living, is the feature which varies in prominence with some apparent eccentricity. On the whole, irregular particles, and especially nutritious particles, lie in a very fluid vacuole of ingestion. Exceptions to both these statements occur, however; thus, minute bacteria have sometimes but scanty fluid surroundings, and the grains of Indian ink may be gathered so closely that the accompanying fluid is hardly appreciable, or may, on the contrary, lie in a vacuole so well marked that Brownian movement is obvious.

<center>INTRACELLULAR PHENOMENA.</center>

The Successive Phases of an Act of Digestion.

The preliminary accumulation of particles or building up of the vacuole of ingestion just detailed is a process which lasts through 25 to 40 seconds in an active animal, and may be drawn out to minutes in lethargic forms; but it is by a relatively sudden discharge from the extreme end of the œsophagus that the vacuole or its anterior half* is intruded into the neighbouring protoplasm. The vacuole is sometimes spindle-shaped, sometimes almost spherical, or it may have any form intermediate between these two extremes (fig. 5K and fig. 7); the spindle-shape is probably to be associated with general functional depression; other modifications in form appear to depend on the size of the particles enclosed. The most common shape (fig. 2B) (broadly elliptical, with acutely drawn anterior and posterior ends) is associated with the most minute ingesta, while larger ingesta (fig. 7, *b*) seem to force a wider temporary rupture in the protoplasm into which they enter. When ingestion is complete, the vacuole does not pause, but

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* In speaking of vacuoles which have been watched from the moment of enclosure, I use the terms *anterior* and *posterior* to indicate respectively that end of the vacuole which was first in progression, and that end which was enclosed last.

performs what I may, perhaps, call a movement of *progression;* that is to say, it passes with a steady, gliding motion towards the basal attachment of the polype. This progression never carries the vacuole beyond the concavity of the band-like nucleus (which body, indeed, seems to define the path of ingesta in the basal region of *Carchesium*), and, dying away, it gives place to a phase of *quiescence* which is generally well marked. The vacuole pauses after turning through one or even through two right angles; it pauses, that is to say, with its long axis at right angles to, or parallel with, the long axis of the polype (figs. 2 and 5).

Up to this point it cannot be said that simple inspection reveals any constant change in the vacuolar contents. Solid particles, when they are minute enough to be present in numbers, are distributed uniformly in the medium which holds them; small Infusoria, if by chance they are enclosed, are active. And although, in some cases, movement at and near the centre of the vacuole ceases, or becomes, at least, less readily appreciable, yet more frequently Brownian movements, or the wider excursions of motile bacteria are obvious still. When, however, the phase of quiescence has persisted for some seconds (in healthy specimens), there is a striking rearrangement of the contents of the vacuole; the change is of such a nature that the solid particles lying scattered until this moment are gathered centripetally, and clear fluid passing centrifugally from among them surrounds the central composite mass. To this phenomenon I propose to apply the term *aggregation,*[*] since its salient feature is the approximation of particles which were separate initially; it is so striking upon occasion, and at the same time so far modified by the nature and disposition of the ingesta, that the following somewhat detailed description seems justified.

Case 1.—*The aggregation is single; one central mass represents the scattered granules of the preceding stages.*

The large majority of the ingesta of *Carchesium* illustrate this form of *aggregation;* at the same time they vary so much among themselves in size, shape, and density, that unity of type in the action is sometimes obscured by secondary modifications. I will choose certain ingesta which seem especially fitted to illustrate this association of fundamental likeness with superficial difference, and will linger a little time to consider the fate of each.

Finely divided Coagulated Proteid.

Carchesium, fed with the coagulation precipitate separated from the diluted white

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[*] It is with some reluctance that, in applying the term *aggregation* to this phenomenon, I use a word to which Darwin has given a different and definite technical meaning in his work on *Insectivorous Plants:* No other word, however, describes with equal accuracy the striking rearrangement of solid particles which takes place so suddenly in a vacuole of ingestion, and, by the light of later work on the secretory activity of plant cells, the description of change in the cells of the tentacles of *Drosera*, given with such faithfulness by Darwin, must be considered, on the whole, as of classical rather than immediate interest. Partly on these grounds, and partly in deference to clearer judgment than my own, I have spoken of the centripetal clustering of ingested particles as " aggregation " throughout the pages which follow.

of fresh eggs, may be regarded as ingesting minute, irregular particles of nutritious matter suspended in water, or rather, in a very dilute solution of salts ; some soluble organic matter is probably present too, when the colony is living in an impure medium. In this case the fluidity of the vacuole of ingestion is generally well marked, the faint pink tint, familiar to histologists as belonging to thin protoplasmic films which are separated by fluid, is distinct during the phases of progression and quiescence, and the dancing particles of proteid are distributed uniformly. But when the phase of quiescence is over, their movements cease; those which lie peripherally leave the boundaries of the vacuole, and, with a general and relatively rapid centripetal motion, the central coherent mass is formed. Nor is this union temporary ; the individuality of the granules is gone for ever, and time and further change only tend to perfect the apparent homogeneity of the composite solid.

This process is seen most satisfactorily with high powers of the microscope,* and the absolute displacement of each solid particle is of course exceedingly small. I find that in no recorded case have I estimated the distance traversed in *aggregation* as greater than $6·2 \mu$, and so marked an excursion as this is rare. The phenomenon is very striking, however ; and, I may add, the difficulty of offering a really satisfactory explanation is great; close observation, if it does not surmount this difficulty, allows one to add the following details to a general preliminary statement.

1. The *aggregation* is almost invariably excentric; I have mentioned that the vacuole of ingestion may turn through one, or even through two right angles before the movement of progression dies away ; clearly then, its most anterior point may pause at any spot along the circumference of a semicircle arching upwards from the concavity of the nucleus (fig. 1, 2a). Yet, however marked the preliminary shifting, it is from the anterior end that the greatest centripetal movement takes place; the mass of gathered granules settles towards that region of the vacuole which entered last from the œsophagus, though even here there is fluid separating it from the surrounding protoplasm.

2. The *aggregation* often begins at one point—not necessarily the most anterior,— but runs round the vacuole so rapidly, that it is only just possible to pronounce the displacement of particles lying along different radii of the vacuole not simultaneous, but successive. And in certain cases the synchronism is perfect, and gives the impression that a force is at work which gathers up all outlying particles, fusing them or establishing a substantial link between them in such fashion that the retreating border of solid matter is not shadowy, but highly refractive and sharp, showing well against the encircling rim of clear fluid (fig. 3k).

3. The first and most striking *aggregation* cannot be regarded as ending for ever all movement of the solid matter involved in it. In the first place, the freshly-formed composite proteid mass is not always homogeneous. It is, indeed, a mass and

not a shell of gathered particles, still, there is sometimes localized admixture of fluid; little rifts, pinkish in tint, hint that tiny drops of fluid have been imprisoned by the first coming together of the irregular particles of proteid. In the second place, actual measurement shows that for some seconds after the first spasmodic clustering the ingested matter shrinks; this shrinkage is gradual, it persists through the next phase, which may be distinguished in the digestive process, and points (as does the gradual disappearance of the drops of imprisoned fluid which have just been mentioned) to a slow but closer gathering of the particles which were moved at first with relative swiftness.

Indian ink suspended in water.

The fate of this form of matter within the substance of *Carchesium* is suggestive, for the particles of Indian ink are extremely minute and practically insoluble in water and saline solutions, and in these points they resemble the proteid precipitate just described. At the same time the nutritious character of boiled white of egg establishes a sharp distinction between the two substances, the clearness of contrast being marked in proportion as each body is unmixed with other matter. The Indian ink may be gathered into the vacuole of ingestion with such energy that the admixture of fluid is hardly noticeable, or it may (and this is more commonly the case) pass through the phases of progression and quiescence of the vacuole, in active Brownian movement. When it is a question of the latter alternative, the Brownian movement is stopped by a centripetal gathering of particles as unmistakable (in vigorous polypes) as the primary *aggregation* of finely divided proteid. Only this difference reveals itself on observation, that some scattered grains of Indian ink are at times left outside in the general clustering and lie peripherally in the expressed fluid, whereas I can quote no instance in which all the solid particles of proteid present in a vacuole of ingestion are not gathered in during *aggregation* to the composite mass which is formed.

When, on the other hand, Indian ink particles fill the vacuole densely, even from the moment of its formation, then the displacement in *aggregation* is not well-marked; but the edge of the enclosed mass becomes sharply defined against a narrow rim of clear fluid which encircles it like an aureole. In no case is there immediate separation of the particles which have come together, despite the absence of cohesiveness shown by Indian ink grains suspended in water; a well-marked composite mass is formed, a mass interesting, as well on account of the fashion of its formation, as on account of its further history within *Carchesium*. The details of this history, however, can be dealt with more suitably later; so, with a simple notice of the facts that the secondary shrinking, so noticeable during the ingestion of proteid, is hardly appreciable when pure Indian ink is enclosed, while the excentric character of the aggregation may be marked, I turn to describe forms of ingested matter which have characters not touched on before.

Particles, nutritious and innutritious, which exceed in size the constituents of the above-mentioned ingesta. Carmine grains, pigment grains (ultramarine), the smaller fatty globules of milk, some bacteria, and small monads.

In Plate 34, fig. 17, b, b_1, I have sketched complex ingesta of carmine and bacteria, and the two diagrams illustrate many of the points which characterize the forms of matter—in some ways so diverse—which I have grouped together here. In the first stage represented (b), *aggregation* has taken place recently, and it is clear that the actual displacement of matter has been slight. In the stage drawn two minutes later (b_1), the contraction, or shrinking, is accentuated, the enclosing fluid is clearer, and the outline of the ingested mass well-defined. These statements hold for most cases in which the vacuole, entering from the œsophagus, is well filled with fairly large particles; the primary rearrangement tends to be unimpressive; it merges into what I have called secondary shrinking, and the whole process is deliberate. When, however, such particles are present scantily—when a few micrococci, or scattered individuals of bacterium termo, are enclosed, then the *aggregation* is striking; and a single spirillum, a single monad, or a single globule of fat, when each is ingested with minute particles, sparsely distributed, may move inwards, quite markedly, from the external limit of the vacuole (fig. 7, a, a_1). The fate of milk globules deserves special notice; I find that the fat of milk is no more available for the nutrition of *Carchesium* than for that of *Amœba* or *Actinosphærium*,* yet the ingestion is eager and persistent. The tiny globules can never, of course, fuse to a homogeneous mass, and (being, indeed, generally densely packed from the œsophagus inwards) they do not, as a rule, illustrate at all vividly the typical spasm of primary aggregation, or gradual following shrinkage. But their undoubted coherence into mulberry-like masses is striking (fig. 7, c), and if it be urged that the proteids of milk in solution (fresh), or precipitated (albumen in boiled milk), may have their share in effecting this union, still the facts seem to demand the existence of some homogeneous unifying substance, its outline joining the peripheral fatty globules, while those that are more internal lie embedded in it. It is natural to try to test the validity of such an inference by reference to the fate of ingested proteid and Indian ink. I am anxious, however, before entering upon any discussion of the meaning of *aggregation*, to increase the list of facts upon which such discussion may be based, and I turn to record the results of some observations of a second main modification of the phenomenon.

Case 2.—*The movement is double; two processes, or, more probably, two steps in one process, may be distinguished, unlike in duration, and, to a certain extent, in result.*

The composite mass is made up of a central portion, shaped by the first rapid gathering, and a cap-like or spherical border of solid substance deposited later (fig. 8, 1d, 2c). In one series of experiments this double movement constantly followed the ingestion of the white of stale eggs coagulated after dilution. The

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* M. MEISSNER, 'Zeitsch. f. Wiss. Zool.,' vol. 46. 1888 M. GREENWOOD, 'Journ. of Physiol.,' vol. 7.

feeble coagulation, which I obtained by boiling, in this case, contrasts strongly with the dense precipitate thrown down by heat from a solution of fresh white of egg. And since it is known that when eggs are kept, albumoses and peptones make their appearance and increase, and that albumen coagulates feebly in alkaline solutions, it seems probable that the opalescent fluid, obtained by boiling the stale "white," offers for the attack of *Carchesium* a constituent which has hardly been considered as yet—nutritive matter in solution. Associated with this soluble food, we have, in the case under consideration, a scarcity of suspended particles, and the relative importance of each factor in producing the phenomenon I am about to describe is not easy to estimate.

The first centripetal movement is a vigorous reproduction of the process as it has been described above. The cluster of proteid particles is excentric in position, its constituent granules, probably because they are scattered initially, come together with much energy, and at the same time may leave among themselves the little fluid-filled rifts described before. This admixture of fluid is so striking sometimes that the ingested body may be termed vacuolate ; its border is always sharp and unbroken ; its size (and *à fortiori* its mass) is less than the ordinary size of a food ball, shaped by recent *aggregation*. This difference is of course a difference of degree, but a qualitative peculiarity becomes obvious almost at once in the fluid of the vacuole of digestion. I have tried to represent this in fig. 8, and to show that after the first gathering of granules slight opacity in the outlying fluid hints at the appearance of fresh solid matter. There is, in fact, a second movement shortly ; little bullæ of clear fluid appear round the edge of the vacuole (fig. 8, 1c), and the opacity of its contents becomes slightly accentuated ; later, a gelatinous mass retracts, with slow centripetal motion, and settles round the "nucleus" formed by the first quick movement (1, *d*). The substance separated thus by the second action is generally homogeneous, and often difficult to see ; in rare cases, the preparatory turbidity of the vacuole, of which I have spoken, is exaggerated to granularity, and when this is so, the shrinking solid is granular too (fig. 8, 2c). I have said that this second retraction is slow, indeed it may be many minutes after ingestion before the double sphere lies fully in clear fluid ; rarely, however, there is an approach to the rapidity of primary *aggregation*. I think that the rate of movement, the extent of retraction, and the consistence of the second shrunken mass depend upon the existence of a certain balance, in the proportion of soluble and suspended matter first ingested, and upon the amount or energy of the substance causing retraction, which is passed from the surrounding protoplasm into the vacuole. Thus, double *aggregation* can be made single by the addition of an overwhelming quantity of finely divided substance to the fluid, of which soluble matter was an important constituent before ; on the other hand, a few inert particles, such as those of carmine or Indian ink, do not hinder the moulding of the double mass, but are recognizable, for the most part, near its centre—they are involved, that is to say, in the first sudden movement.

Again, the degree of the second retraction varies; as a rule the shrinking is so marked that in the stage of *storage** the double character of the ingesta is hardly perceptible; more rarely there is a loss of vacuolar fluid, while yet a granular "nucleus" lies clearly within its gelatinous investment—the mechanism of retraction has failed partially, either because of unusual lack of vigour, or because of excessive resistance in the substance which is to be moved.

Any specimen of *Carchesium*, in which this double clustering of ingested matter is demonstrable, can, as I have said, be induced to perform the single movement by suitable change of food; it would seem, then, that we are dealing rather with inconstant stimuli than with inconstant response of cell substance. And the whole variation is valuable, mainly, I think, because the slower changes which characterize it are of help in the attempt to analyse the quicker, usual movement.

The somewhat detailed description which I have given of this phenomenon of *aggregation* may tend to obscure the fact that it is, in *Carchesium*, but one phase in the sequence of digestive change; that all the important transformations immediately bound up with solution of food are yet to follow. It ends the period of quiescence, during which most ingesta tarry within the basal curve of the nucleus; and it precedes a second mimic peristaltic movement, which, since the ingesta pass once more towards the peristome (though on the side of the polype opposite to that on which they entered), may perhaps be distinguished as the movement of *retrogression* (fig. 2, B, *a*, *b*). This is slower than the initial *progression* from the œsophagus, and is usually characterized by the slight continued shrinking of the ingesta on the one hand, and, on the other, by the enlargement of the vacuoles which surround them. These changes are indicated in fig. 2, A, B, fig. 7, *b*, *b*, and, though they vary under changed conditions, may be said to accompany the transit of all particles, whether they are relatively large or minute, insoluble or nutritious. But when *retrogression* has carried the vacuolate ingesta almost to the level of the peristome, there are indications of varying fate; and the cell-substance of *Carchesium* shows that discriminating reaction to the nutritious or innutritious character of enclosed matter for the reality of which I have pleaded before in the case of *Amœba*.

Nutritive ingesta, following the course of the red line in fig. 1, pass towards the more deeply-seated substance of the animal (I speak now of typical acts of digestion), and may pause at any point in the area shaded with transverse lines, or may move through it slowly. In either case they lose the fluid which surrounds them, and with its disappearance there is a total intermission of digestive activity; the ingesta pass into what I would call the stage of *storage*; this may persist even for hours. Throughout this phase the enclosed masses, which have origin in coagulated proteid, are smooth, and oval or spherical; many ingesta, not of distinct experimental origin, and therefore doubtful in nature, form rather dense wrinkled masses, and in clusters

* *Vide infra.*

of ingested bacteria the individuals can still be distinguished as bright points, or (after proper treatment) as deeply-stained dots. Ingesta, whatever their nature, have reached the extreme point of solidity, of shrinkage; and a polype, after vigorous feeding, is chalk-white by reflected light, and, by transmitted light, studded with relatively opaque patches, however transparent may be its own cell-substance.

Eventually, however, the storage stage is ended;* there is no constant or noticeable locomotion of the ingesta, but vacuoles re-form around them at any point over a large area. And this freshly-secreted fluid has a powerful solvent action on proteids; even coagulated proteid succumbs, and the semi-mucilaginous investment of bacteria does not guard them from speedy loss of discreteness. In figs. 3 and 4 diagrammatic representations of this part of the digestive process are given, the ingested matter being coagulated proteid in fig. 3; in fig. 4, bacteria.

In fig. 3 one food-mass out of many is chosen for reproduction, and the first sketch is made immediately after *aggregation* is accomplished. The movement has been inconsiderable, and only a small amount of fluid surrounds the composite mass in whose substance there is just the indication of tiny drops of imprisoned fluid. At a, the stage of storage is reached; the fluid of the vacuole, separated out by *aggregation* and developed more clearly during the movement of retrogression (of which there is no picture here), has disappeared, and the proteid sphere is denser and more clearly defined than at any other moment during its intra-cellular career. b, c, and d represent stages in development of the digestive vacuole, and solution of its contents. At e, the proteid matter is obviously reduced in amount; the vacuole holding it is still very large, and has approached the area from which discharge takes place.

Fig. 4 differs in two points only. In the first place, the actual enclosure of the ingesta was not seen; it is not certainly there, but only (from comparison with known cases) probable in a high degree that they are clustered masses of bacteria; in the second place, the time data given here help one to realize the vigour of digestive action, even on enclosed matter which does not promise ready solution. It will be seen that in less than an hour after the first formation of a digestive vacuole (a) there is marked reduction of the food-mass attacked, while at the same time no scattering of its substance can be made out. These two features characterize every energetic digestive act, but they vary in prominence. Thus, if many insoluble particles are bound together by but a slight admixture of nutritious matter, then in the later stages of digestion such particles may be set free; this is often the case when Indian ink is ingested with proteid (fig. 5, a), and may be seen rarely when *Carchesium* is fed with milk. Or, again, the reduction in size may be inconspicuous when the insoluble matter mixed with any food-stuff is bulky, or is so constituted that no close packing of its particles is possible (fig. 7, b, b_1).

I should like to emphasize another change which is at least as constant an accompaniment of digestion as continued cohesion of food particles or reduction in size of

* Some actual time details are given later.

the mass which they compose—I mean increase of transparency, loss of solidity and of firm outline. I have tried to represent this in the figures of *Carchesium*, but with slight success, for in reality it is so striking that there is but little tendency to confuse ingesta before and after the stage of storage, though in both cases (typically) they are vacuolate. Indeed, as it has been pointed out that after the first loss of vacuole the extreme point of concentration is reached, so I may add that never is there such shadowy outline, never such sharp contrast in refraction between accidental insoluble particles and the apparently mucilaginous basis by virtue of which they cohere, as in advanced digestion.

Complete solution of ingested matter is extremely rare in *Carchesium*, especially when it is a question not of the relatively pure nourishment provided by experiment, but of the mixed diet of a struggling life; after a varying time,[*] then, insoluble remains of all kinds pass from the more central position where the digestive ferment has attacked them (fig. 1., solution) and come to lie in what I have called the area of *discharge* (fig. 1, discharge). According to GREEF,[†] all fæcal matter is expelled into the pharynx (at about the junction of its external and middle thirds) from a ridge, running transversely to the long axis of the polype in this region, and round about this narrow area the vacuolate food masses are grouped when digestion is at an end. And here there is occasional fusion of vacuoles, so that two or more ingesta are placed in common fluid surroundings (fig. 6, *d*). This phenomenon may, indeed, occur at any period in the digestive cycle; thus, in fig. 6, *c*, I have represented a fusion of four ingesta effected during *retrogression*, and the fusion of vacuoles of *ingestion* is not infrequent. But the coalescence is rather to be associated typically with advanced digestion than with these early stages, and recalls the fashion in which, in *Actinosphærium*, many ingesta may be passed to the exterior from a common excretory vacuole.[‡] In *Carchesium* the occurrence is rarer, and it may, moreover, be obscured by a characteristic want of synchronism in the ejection of fluid and solid matter; as a rule, nutritive ingesta reach the area of discharge in well-marked vacuoles, but these are almost invariably reduced in size and may disappear before extrusion of the solid matter (fig. 3, *e.*). The assumption that the fluid lost thus as a discrete vacuole does, indeed, pass from the animal, and not into the protoplasm which secreted it originally, may be regarded as hasty, and so bewildering are ciliary movements and the constant shifting of the whole polype that the fate of anything as inconspicuous as is fluid tends to be obscure. But apart from *à priori* considerations, which lead one to assume the existence of waste matter here, the somewhat sudden character of the disappearance and an occasional preliminary ejection of free granules or viscid flocculi serve as some direct evidence of outward movement. After the diminution or disappearance of its fluid surroundings the solid residue is discharged, passing as if slipped into the

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* *Cf.* below, p. 372.

† R. GREEF, *loc. cit.*

‡ M. GREENWOOD, 'Journ. of Physiol.,' vol. 8, p. 283.

pharynx. Here it pauses until such time as some of the currents called into being by the vigorous action of surrounding cilia sweep it to the exterior.

I have said in an earlier part of this paper that **innutritious matter** is swallowed by *Carchesium* with undiscriminating eagerness, if its constituent particles are small. Indian ink grains and coagulated proteid mixed equally in the surrounding water give rise to ingesta, in which both substances are mingled impartially. Further, it is quite usual in vigorous polypes to get a replacement of one set of ingesta by others of unlike nature, when the suspended particles proper to each group are made to preponderate successively outside the animal. And in experimenting thus, I have never been able to note any clearly selective ingestion ; coagulated proteid may replace carmine or bacteria, and may be ousted in turn by Indian ink or milk ; it is the quantity or distribution of matter that determines its entrance rather than its availability for subsequent digestion. Even after enclosure, the onward movement of progression is characteristic, and, the phase of quiescence being ended (usually) by a typical movement of aggregation, retrogression follows. Finally, after a pause in the " area of *solution*," discharge takes place from the anal ridge. Only in the duration of these several events, and in the really important details, the wrappings to this bare thread of movement, can we trace the fact that innutritious matter does not stimulate the cell substance of *Carchesium* so effectually as does digestible food.

In the first place, the time of enclosure is short. I will not anticipate the more detailed statement on this point which is to follow, but will only say that this diminution does not affect the first periods I have distinguished. The time taken up by progression and quiescence is, on the whole, very slightly longer than when true food stuffs form the ingesta. But sojourn in the area of solution and the area of discharge is shortened, and 40 to 60 minutes after the first enclosure, ejection may have been even considerable.

In the second place, it is noticeable that very many non-nutrient ingesta are discharged without that re-formation of vacuole which marks the actual solution of digestible matter. Deprived of fluid after the movement of retrogression is over, they acquire no fresh fluid from the surrounding protoplasm, and they are set free finally as spherical or more commonly elliptical masses (fig. 2, A, fig. 16, *d*). But I cannot leave this statement, contrasting as it does with the history of true digestion, without a modification which at first sight is damaging to its effect. It happens sometimes that ingesta, moulded from insoluble pigments, never show complete loss of the vacuoles which have been brought out by *aggregation*. Not only does the fluid persist, it increases in amount, and in such case the peripheral grains of pigment (I speak now of finely particulate matter) are freed into the fluid of the vacuole and show typical Brownian movement (fig. 5, *a*). There is, however, in no case thorough disintegration of the ingested mass ; whatever change sets free the granules is circumferential only, for a central solid remains and shows on ejection no lessened

cohesiveness. This phenomenon is not extremely rare, but may be termed exceptional. I hope to discuss later, its relation to the view formulated above, that the secretion of the true digestive fluid is selective, and to show that the two are apparently rather than really discordant.

The length of time during which Carchesium retains ingesta, and its distribution among successive phases of digestion.

When a group of polypes is left overnight in water holding coagulated proteid in suspension, it is usual in the morning to find each polype crowded with ingesta in the stage of storage (Plate 34, fig. 2, *C.s.*). And this state of things may persist yet longer if the animals and their surroundings are left undisturbed, whereas, mounted and watched by transmitted light, they soon show signs of digestive activity; vacuoles are formed round some of the ingesta at least, and the changes which I have described above as characterizing solution, follow. On the other hand, this long enclosure is by no means inevitable, and digestion may follow ingestion very rapidly. Thus in fig. 3, in which actual taking in of boiled white of egg went on under the microscope, the stage of storage (*a*) was but of short duration, for all the digestive changes which succeeded it (figured at *b, c, d, e*) were sketched in little more than one hour from the time when ingestion began. It may even be that there is no suspension of digestive activity at all; the fluid which the movement of aggregation defines is never lost in this case; on the contrary, it increases in amount as digestion proceeds, and the assumption that its constituents change seems justified. The promptitude with which secretory activity may manifest itself under changed conditions is represented in fig. 4, and this figure and fig. 2 c. further serve as illustrations of that temporary unlikeness in the fate of like ingesta to which reference has been made above. Digestion may be localized at first with apparent caprice; some food masses are taken and others left, though there is no final failure of activity but only delay.

It will be gathered from these statements that there is some difficulty in fixing within narrow limits the time during which *Carchesium* retains its nutritive ingesta; it would seem indeed that many factors cooperate to produce variations in the onset and duration of true digestive activity, and that the characters of the ingesta and the varying condition of the animal are equally potent with changing external conditions.

Among external conditions, however, there can be little doubt, I think, that **light** has a stimulating action. It is true that the necessary limitations of a " hanging drop " suggest that other influences, less readily appreciable, act upon specimens that are being watched under the microscope; still when due care is paid to irrigation and aëration, one is impressed by the constancy of a certain sequence of events. If the ingesta are in an advanced stage when observation begins (this happens with recently caught *Carchesium* which often holds greatly changed bacterial food) then there is speedy *discharge*; food is digested, or it may be, hurried to premature ejection when the animals are mounted during the *storage* intermission of secretory activity. Light

does not produce appreciable lesion in the substance of *Carchesium;* the nuclei are unaffected, and even under artificial light* there may be vigorous ingestion: rather, it stimulates to advance in the digestive cycle,—to secretion, or it may be to ejection.

Apart from the influence of light, the presence of fresh food material has some effect on the attitude assumed by *Carchesium* to foreign matter already within its substance. It is too much to say that ingesta of one kind will replace those of any other kind, but ejection always appears to be promoted by fresh ingestion, especially when the preexisting ingesta have been enclosed for some time. In the case of *Amœba* I noticed the tendency to reject carmine or grains of starch when such highly nutritive ingesta as monads became available, and a slightly different adjustment of the same complementary acts, shows itself in *Carchesium* in an increased tendency to eject débris when many fresh ingesta are being formed. The difference consists in this, that nutritive matter is not clearly prepotent, at least in vigorous animals; the expediting action of renewed ingestion on (*ex. gr.*) the discharge of bacterial remains is as obvious when the newly enclosed matter is Indian ink, as when it is finely divided proteid.

Last among the external influences which affect the duration of enclosure I am inclined to place mechanical stimulation, such disturbance, that is to say, as is bound up with sudden change of environment and with manipulative interference. This is not often dissociated from change in illumination, and for this and other reasons I am ignorant of its true importance. But ejection sometimes begins synchronously with remounting, manifesting itself with a vigour which lessens presently, and I often had occasion to associate the discharge of effete matter in *Amœba* with mounting or transference of the animal; it seems difficult, then, not to regard some obscure contact stimulus as one more complication tending to hasten discharge.

It remains to consider the relation of ingested matter not to variation in external conditions but to the state of *Carchesium* at any moment. It is readily conceivable that, changing in condition, the animal would react changingly to constant stimuli, and that in this fashion variations in the manifestations of digestive activity would occur. Any attempt to estimate the value of this factor, however, must follow an examination of the way in which variation characterizes not only the whole time of enclosure but each succeeding phase.

1. *Extra-cellular phenomena.*—In an earlier part of this paper I described the way in which particles are gathered into the pharynx and gullet of *Carchesium* to aid in forming the vacuole of ingestion. If we disregard the wide intervals which may separate successive periods of feeding, and take no account for the moment of partial and ineffective accumulation of solid matter in the œsophagus of a lethargic form, it

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* Any specimens examined by artificial transmitted light are exposed to rise of temperature in the medium in which they lie, as well as to intense illumination. I have found that signs of lesion appear very constantly with any considerable rise of temperature, but below this point I can draw no clear distinction between the effect of light and that of heat.

becomes clear that there is considerable regularity in this preliminary act. Such variations as occur belong rather to different polypes at different times than to any one animal during one period of feeding. In vigorous ingestion the time which passes between the internal discharge of two succeeding vacuoles is usually 40 seconds; greater energy shortens the interval to 30 seconds, and in some cases, which are yet quite healthy, it is drawn out to 1 minute or rather more.* And since there is practically no intermission of ciliary activity, the time which separates the acts of ingestion may be regarded as measuring, too, the creation of the vacuole which is to move. Whilst watching any one polype I have never seen a sudden confusion or exchange of these time limits, such, for example, as an adoption of the 30 seconds interval for one of 1 minute; the rhythm is approximately regular, but in different animals its rapidity varies.

2. *Progression.*—It will be remembered that the position taken up by the vacuole of ingestion during *quiescence* is not quite constant, in other words, there is a journey of variable length through the substance of *Carchesium* before progression is at an end. And the time spent in this journey varies, but not, I think, directly with the extent of locomotion; it may be that a vigorous impulse effecting ingestion lasts long, carries the food mass far and moves it rapidly, and that a faint impulse acts sluggishly and fails soon. I give 10 seconds as the characteristic duration of normal *progression*, but the time may be lengthened to 14 seconds, or may be shortened to 5 or 6 seconds.

3. *Quiescence.*—The phase which follows progression is, to a certain extent, its complement; thus, it lasts typically for 9 seconds, may be shortened to 5 seconds or lengthened to 25, the variations tending to be inversely as those of the movement of progression. The correlation is incomplete however, so that the total time elapsing between *ingestion* and *aggregation* is somewhat inconstant; it may vary from 11 seconds to 37 seconds, but is commonly 20 to 24 seconds.

4. *Aggregation.*—Primary *aggregation*, when it is vigorous, is practically instantaneous. It is one of the most constant periods in this drama of digestive change, and among some hundreds of observations I have records of hardly more than a dozen which show any marked divergence on this point; $\frac{3}{4}$ second, $\frac{4}{5}$ second, or even 1 second represent the most frequent degrees of extension. I have one case in which $1\frac{4}{5}$ second passed before *aggregation* was achieved, and yet another case is recorded in which the lethargic character of the movement was so pronounced that I leave it for special consideration later.

5. *Retrogression.*—When primary *aggregation* is over, the digestive vacuole pauses for a variable time before the development of the slow retrogressive movement, and it is not easy to see how the impulse to retrogression arises. A certain displacement would, of course, result from the recurring arrival of fresh vacuoles of ingestion, but this movement is determinate in direction, and its onset does not necessarily coincide

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* R. GREEF describes successive acts of ingestion as occurring after an interval of a quarter of an hour.

with the advance of new food material. In periods of vigorous feeding ingesta are passed back and reach the area of solution in 1 minute; this time may be doubled in animals which are apparently quite healthy, and, as I have tried to make clear in another place, the path is sometimes shortened, and sometimes shows irregular extensions of which no account is given in the purely diagrammatic scheme of fig. 1.

6. *Storage.*—Passing into the large central area where food is stored as well as digested, the ingesta of *Carchesium* enter on the most variable period of their sojourn within its substance. After gradual loss of the fluid which first became obvious during *aggregation*, they may lie for 12, 18, or 20 hours showing no sign of change; on the other hand, I have seen vigorous re-formation of a vacuole, and the onset of solution only 30–60 minutes after the first loss of fluid. This shortening of the storage stage is associated with digestion under continuous observation, and finds its extreme expression in the entire omission of any pause and loss of vacuole; *solution*, in this case, follows *retrogression.*

7. *Solution.*—This phase, which may be regarded as giving point to all the preceding ordered marshalling of ingesta, is much more constant in length than is the stage of antecedent storage. It is obvious that the matter upon which the fluid of each vacuole has to make its attack varies in intrinsic digestibility, in admixture with innutritious foreign particles, and in amount and density. Thus, there is undoubted qualitative difference between the substance of bacteria and coagulated white of egg. Without attempting any rigid definition of the time limits of the solvent process, then, I will only say that characteristic transparency of the ingesta is unmistakable 15 or 20 minutes after the vacuole has re-formed, and that within an hour the change in aspect—often with great reduction in size— is very marked. In like manner, when the storage stage is omitted and we deal not with a freshly-formed vacuole, but with enlargement of one pre-existent, and (presumably) with variation in its constituents, signs of solution are striking within an hour.

8. *Ejection.*—As forming the last period in this history of intracellular phenomena, I group together the actual rejection of matter and its antecedent stay in what I have called the area of discharge. The preliminary pause is sometimes long and the transference of the débris of ingesta from the area of solution may be slow and irregular, even when all appreciable solution is at an end. Hours may, indeed, intervene here, while, on the other hand, I have seen the much changed remains of nutritive ingesta discharged within 1½ hour after enclosure. This is the stage which is shortened most readily by mechanical, and possibly by chemical stimuli; it is indeed not uncommon when disturbances of such a nature are set up, to see mucilaginous débris, apparently the accumulation of hours, discharged in irregular succession. The act of ejection is rapid, but each mass of waste matter is passed out into the pharynx without any constant accompaniment of fluid, and may linger there for some seconds, even for minutes, before it is finally set free.

Up to this point I have been speaking of the fate of nutritious ingesta with or

without admixture of innutritious substance; the case in which the nutritious element is minimized,—it may be, abolished, needs one word of description. When carmine grains, particles of Indian ink, or of ultramarine blue are taken in by *Carchesium*, there is no appreciable change in the first responsive events, and to the end of the movement of retrogression the time relations of the different periods are practically unaltered. Later, however, two divergences from the programme I have just sketched are striking. In the first place, the whole time of enclosure is shortened. This has been stated in the general description of the fate of innutritious matter given on p. 367, and I will merely add, in the first place, that constant observation, mechanical disturbance, and all those conditions which tend typically to abbreviate the digestive cycle are especially potent here and often promote vigorous discharge. Ejection may indeed be considerable, 50, 40, or even 35 minutes after the beginning of ingestion. In the second place, it is mainly the stages which correspond to *storage* and *solution*, which are curtailed or obscured. I have before alluded to the fact that there is unusual persistence of fluid in rare instances, but these can hardly be regarded as exceptions to the statement that loss is not followed by renewed secretion; that there is no active re-formation of vacuoles if the direct descendants of the vacuoles of ingestion disappear. In the great majority of cases the pigment masses lie as if stored until they pass to the area of discharge before ejection. And here the time relations of succeeding acts approximate again to those which characterize the end of true food stuffs. There is nothing distinctive in their journey to the area of discharge nor in the act by which they are ejected.

In the following Table I have gathered together most of the numerical results which have just been discussed; it will be seen that rather pronounced variability interferes with definite statement in some cases; I have therefore given, where it is possible, the time which I regard as belonging typically to each event, and the extreme variations from this, which I have recorded.

	Total time of stay in substance of *Carchesium*.	Extra-cellular gathering of ingesta.	Progression.	Quiescence.	Aggregation.	Retrogression.	Storage.	Solution.	Ejection and preliminary stay in area of discharge.
Shortest time recorded	30 mins. (innutritious matter) 1 hr. or 1¼ hr. (nutritious matter)	30 secs.	5¼ secs.	5 secs.	Instantaneous	50–60 secs.	Omission or 30 mins.	50 mins.	10–20 mins.
Longest time recorded	30 hrs.	65 secs.	14¼ secs.	25⅝ secs.	1¼ sec.	? 5 mins.	? 22 hrs.	...	Some hours
Typical time recorded	...	40 secs.	10⅜ secs.	9·04 secs.	Instantaneous to ¾ sec.	1 to 1½ min.	Some hours		

The significance of some of the phases through which ingesta pass in the substance of Carchesium.

The meaning of aggregation.—The striking feature of this phenomenon is undoubtedly the movement of suspended particles, the fundamental feature is the existence of a force which moves them—a force constantly centripetal, though not always perfectly symmetrical in action, and operating instantaneously in times of vigour.

The constantly sharp outline of the mass of moving particles, whatever the actual displacement of its constituents, the persistent central cohesion, not only of possibly glutinous matter, but of oil drops or grains of Indian ink, and an occasional lack of synchronism* in action, forbid the hypotheses that aggregation is due solely to the centripetal discharge of fluid into the vacuole from the surrounding protoplasm, or to the separation and contraction of a highly elastic lining film. On the other hand, all these facts are in harmony with the view that the phenomenon is essentially one of shrinking—that there is rapid retraction of some viscous matter which entangles in its substance, and thus aggregates any solid particles present in the vacuole of ingestion. This hypothesis finds further support in the considerable staining power of food masses formed from coagulated proteid, as contrasted with the reaction of the proteid particles before ingestion, in the persistent shrinking exhibited by ingesta until they are stored, destitute of vacuoles, and in the phenomenon of double *aggregation* (*v. sup.*). And certain secondary modifications of the movement, which are observable at times, offer no hindrance to its adoption. Thus, in some specimens of *Carchesium* which are apparently lacking in vigour, a food mass formed by *aggregation* may cling for a time to the walls of the vacuole by delicate viscous threads springing from, and finally retracted to, its poles (fig. 6, *b.*); at other times so much fluid is entangled in a food mass as it forms that it is clearly, if temporarily, vacuolate, and in yet other cases the central particles contained in a vacuole of ingestion may show lingering movement, while retracting hyaline substance separates them from the periphery of the vacuole. These modifications occur rarely, and I regard each as an expression of partial failure in the action of the mechanism which underlies *aggregation*; there is, as the case may be, localized delay in the retraction of the viscous matter, or localized lethargy in its maturation.

The Origin of the Retractile Substance.—The formulation of this hypothesis as an interpretation of the phenomenon of aggregation leads naturally to the darker problem; how and where is the retractile substance formed? The continued ingestion of rich food by *Carchesium* does clearly increase the granularity of its cell substance, but the converse activity of secretion brings about as little undoubted

* In fig. 6, *a*. a_1, a marked instance of this intermittent action is illustrated; after most of the particles were stilled, Brownian movement persisted at the poles of the vacuole; finally, the last vagrant granules were gathered in, forming cap-like additions to the main mass.

structural change here as in the case of *Amœba* or *Actinosphærium*.* A polype, in which fifty successive acts of *aggregation* have been observed, does not, save for its abundant ingesta, differ from one that is fasting. It is rather on *à priori* grounds, then, that I am inclined to localize the formation of viscid matter in the period or periods which precede *aggregation*. The period of *quiescence*, which has so dramatic an ending, is in itself devoid of obvious event; there is then too great readiness, perhaps, to associate with it, in thought, some phasic heightening of obscure molecular activity. As a matter of fact, *quiescence* tends to vary inversely as *progression*, in other words, a fairly constant interval elapses between the moment of ingestion and the aggregation of ingested particles. And, throughout this interval, there is typically continuation of " proper " movement or of Brownian movement with undiminished energy, though rarely I have seen considerable excursions of the peripheral particles in a vacuole round an apparently viscid central mass. I conceive that the retractile substance does not, when first formed (possibly immature), offer an effective hindrance to the oscillation of fine particles or the movement of small organisms, that it may vary in amount and condition, thus offering a varying resistance to movement, and that it is probably accumulated in the vacuole of ingestion during the period of quiescence, although the possibility of earlier formation cannot be excluded.

Lastly, it may be asked what initiates the act of shrinking? Why does the viscid substance in its first retraction move with such vigour? I would suggest that the phenomenon is a modified clotting action. In all perfected clots there is extra-cellular interaction of two bodies, or it may be, reconstitution of one body, and with the chemical change physical change is associated—a separation of solid matter, varying somewhat in character, a subsequent shrinking, more or less pronounced.

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* I may add that here (once more, as in the case of Rhizopods) the data are lacking yet, which would enable me to correlate any change in the rhythm of the contractile vacuole with the waxing or waning of digestive activity. All the observations which I have made lead me to associate incompleteness or paralysis of the characteristic pulsations with such lesions of nutrition as occur when aëration is deficient, or when *Carchesium* is poisoned with carbonic acid, that is to say, with phases in the metabolic cycle more remote than those concerned with the solvent processes of digestion. I hope at some time, and after more experiments, to discuss this point.

To any one familiar with the structural characteristics of the *Protozoa*, it will seem gratuitous to insist on the sharp contrast which the phenomenon of *aggregation* offers to the rhythmic movement of a *contractile vacuole*, so little have the two processes in common. I have pointed out in a former paper (' Journal of Physiology,' vol. 8, p. 264,) that in *Amœba* " the true digestive vacuole shows no sign of contractility," and the statement holds in the case of *Carchesium* without qualification. The gathering together of ingested particles, which is so striking and so sudden in *aggregation*, only throws into clearer relief the unbroken line of cell-substance bounding the vacuole; on the other hand, the typical rhythm of the *contractile vacuole* owes its very existence to the spasmodic approximation of the vacuolar walls, which advance, obliterating for the time the appreciable fluid-filled cavity. A *contractile vacuole* is, without exception, destitute of foreign enclosures; the process of *aggregation* is bound up inseparably with the movement of recently ingested solid particles.

Some evidence has been brought forward for the belief that such a separation of solid matter takes place in the digestive vacuole of *Carchesium*, and it may be conceived that there is an extra-cellular (but intra-vacuolar) discharge of matter which clots and which is predestined, by virtue of its proper constitution, not to gradual increase of viscidity, but to a quick shrinking, almost synchronous with its first deposition, and as obvious as the other contents of the vacuole will allow. It may be said that this conception is too hypothetical to be of value, but I would urge that the contrast between the fluidity which precedes *aggregation* (in the vacuole of ingestion) and the viscidity which it initiates is very sharp, and that the quickness of retraction—no necessary part of typical clotting—may be determined to some extent by a new factor in the vacuolar fluid. When delicate indicators of the presence of acid* are given to *Carchesium* the maximum change of tint which they show is associated with the stage of storage; the onset of change dates from the phase of quiescence and becomes more marked during the movement of retrogression. In other words, the acid reaction of the digestive vacuole is perceptible about the time that *aggregation* is accomplished, and though I have failed to detect an increase of size in the vacuole at the instant when movement is striking, it is clear that a secretion of fluid, even important in character, might, if diffused over the surface of the vacuole, affect any one diameter but slightly, or that localized secretion coexisting with localized absorption might leave the total bulk of accumulated fluid unchanged. Bearing in mind the points which have been mentioned more than once, the sharp demarcation of aggregated matter from the fluid which surrounds it, the onset and occasional accentuation of change at the periphery of the vacuole of ingestion, I venture to put forward the view that the acid fluid does not act by sharing directly in the stilling of motile ingesta, which is so characteristic here, it rather heightens the instability of previously secreted matter, so that clotting is induced.

The whole mechanism of retraction, then, may be thus conceived. At the moment of aggregation, substance which has been secreted during the phase of quiescence, probably in an immature condition (*i.e.*, as immediate antecedents of the final body), undergoes a change which may be regarded as a specialized form of clotting. Shrinking as it clots, it entangles solid particles which lie near. At the same time there is an access of acid fluid to the vacuole of ingestion, and it seems probable that this change of medium is advantageous to the effective retraction of the clot or even helpful to its first formation.

The Biological Value of Aggregation.

It remains to consider the relation borne by the phenomenon which I have described

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* The presence of acid in the digestive vacuoles of *Protozoa* has been proved by METCHNIKOFF ('Ann. de l'Inst. Past.,' 1889) and LE DANTEC (*Ibid.*, 1890). I have discussed its relation to the process of digestion in *Carchesium* in a paper which is yet unpublished.

to general protozoan digestion.   Is it, we may ask, peculiar to such a highly specialized infusorian form as is *Carchesium*, or is it raised from the level of the merely interesting, to be dignified as a forcible illustration of some fundamental and often hidden process ?   It is not easy to offer direct experimental evidence in favour of the latter view, but the body of indirect evidence which tells in its favour is not inconsiderable.

1. In records which describe the digestive process in the *Vorticellidæ* there is unanimous reference to the spherical masses of food which, circulating in the endoplasm, are so striking optically ; and GREEF* in his " Investigations into the Natural History of the *Vorticellidæ*," gives the following brief account of their formation in *Epistylis flavicans*.   The freshly ingested food mass is spindle-shaped, and passes towards the base (attached extremity) of the body, arching round again to pass anteriorly (orally) to the level of the point of ingestion.   Here a little knob arises at the pointed end of the mass, and immediately the whole is gathered together to a spherical lump.   Other observers are even briefer in description ; the variations in size of ingesta, the presence or absence of encircling fluid, the elliptical shape of the vacuole of ingestion, all these are noted or form the subject of discussion, without any minute account of the details of successive phases.   It is clear, I think, that the ingesta have been observed, as a rule, after *aggregation* is over, but that the statement given by GREEF is really an abbreviated account of the actual process in *Epistylis flavicans*.

2. In Infusoria, other than the *Vorticellidæ*, the references and descriptions also deal with ingesta, in which the welding process is complete.   Thus EHRENBERG† based his celebrated " polygastric theory " on the very general occurrence of " Magenzellen," describing them in forms as unlike as *Monas* and *Stentor*.   It has long ago been shown that the " Magenzellen " are spherical food masses ; that the method of their formation has been left unrecorded is hardly surprising when we remember that these infusoria are often vigorously motile and sometimes opaque, and that details of the process of aggregation have been lacking hitherto, in a transparent, stationary form like *Carchesium*.

3. In discussing the formation and history of digestive vacuoles in ciliate infusoria, BÜTSCHLI‡ draws a distinction between those animals which ingest relatively large food masses and those in which particles, generally minute, are swept into the pharyngeal tube by ciliary action.   He points out that the concomitant ingestion of water is obvious in the latter case, and the vacuoles, in which ingesta lie, are distinct ; when the food masses are large, fluid surrounds them clearly only some time after the act of ingestion.   I refer to the distinction thus drawn, because it throws some light on the early history of ingesta in *Rhizopods*.   In these animals there is no disturbing

* R. GREEF, *loc. cit.*
† C. G. EHRENBERG, 'Die Infusionsthierchen,' Leipzig, 1838.
‡ O. BÜTSCHLI, " Protozoa " (BRONN'S ' Klass. u. Ord. d. Thierr.').

excess of motility, indeed, the act of ingestion has been described in some detail; but there is, I think, no published account of the process of aggregation. I would urge, however, that there is formation of a viscous substance, which unites scattered ingesta during their stay within *Amœba*, and that its presence, hidden by the fact that the food here is often massive, is indicated by the following experimental results: (*a*) In watching the process of digestion in *Amœba*, I have often seen the enclosure of such active infusoria as monads. And in the notes on these observations (made some years ago*) I find frequent mention of the fact that a monad continued to move in the vacuole of ingestion for 5, 7, or 10 minutes, and thus was suddenly and finally reduced to quiescence; the statement seems to afford a parallel to the sequence of *progression, quiescence,* and *aggregation* in *Carchesium* (2). (*b*) Again I was struck, even at that time, by the cohesion of ingesta during solution. I may perhaps illustrate this by reference to an experiment in which a monad and a green protococcus were received into one vacuole of ingestion. The complex mass was watched for six hours, and, after the first onset of change, there was no break in the union of the two bodies; *e.g.*, at the end of the third hour the monad "found a small semicircular mass, fitted as by some force over one part of the circumference of the protococcus;" at the end of the sixth hour the relative position of the two bodies was unchanged. (*c*) Lastly, I may say that this cohesion was not confined to true food stuffs, but probably characterized such insoluble particles as grains of carmine and Indian ink. Thus I described the finer granules of those bodies as moving after ingestion "not freely, but gathered into groups by a basis of hyaline substance which is generally spherical and often difficult to observe." It seems not improbable that it was a secretion and subsequent retraction of viscous matter quite comparable to that formed by the protoplasm of *Carchesium*, which gave the "basis" its spherical form and united the scattered grains of pigment.

If this indirect evidence be allowed to have weight, it supports the hypothesis that the secretion of a viscid retractile substance round ingesta is a widely spread process in *Protozoa*. The *Coelenterata*, where the digestion is localized for the most part in the body cavity, show, as is well known, marked gland cells in their endoderm, and the digestive fluid apparently takes origin in the intra-cellular solution of secretory granules; in *Protozoa* no such formed secretory products can be detected in the substance of a resting cell; the outpouring of the body, which is destined to clot and shrink, is coextensive with their absence.

It is possible, I think, to examine still further, the relation of aggregation to the whole process of digestion. In an earlier paper I have advocated the view that, while the ingestion of solid matter is promiscuous in *Amœba*, the later history of ingesta varies according as they are nutritious or insoluble. In the latter case, they lie in the

' Journ. of Physiol.,' vol. 7, p. 269.

endosarc, not surrounded by fluid ; nutritious bodies, on the other hand, are digested by the fluid poured around them.   In *Carchesium*, nutritious matter is aggregated, bathed in acid fluid, and then, by gradual loss of surrounding vacuole and continued shrinking, reaches its maximum density in the storage stage.   Later, it swells under the action of freshly secreted fluid until it is difficult to draw the line between swelling and solution, and possible to pass from the fluid periphery of a digestive vacuole, through increasing viscidity, to a fairly solid central nucleus.   It is after the secretion of this second fluid that food stuffs are dissolved.   Innutritious matter shows quite comparable accumulation of fluid and subsequent loss with maximum approximation of its particles, especially if ingestion be carried on in the dark.   Mounting at this stage provokes ejection, but not, as a rule, fresh secretion of fluid.   In fact, the great majority of experiments support the view that the cell substance is, on the whole, discriminating in its secretion of fluid with which the solution of food is immediately associated, but that the formation and clotting of viscous matter in the vacuole of ingestion is called forth by ingesta of all kinds.

Since the food ingested naturally by *Carchesium* is very largely made up of bacteria, and may therefore be actively motile, it is conceivable that there is need of some speedy and effective action on the prey, and that this necessity has concentrated the secretion of the potent retractile substance and generated the habit of uniform response to all ingested matter.   On the other hand it may be that indiscriminate aggregation is arrived at in quite another fashion ; the viscid matter is so intimately related to the actual process of digestion, that no entering vacuole, whatever its contents, is sent on unprovided with its share of so important a factor.

Either of these suppositions is in harmony with the last point I wish to mention as suggestive of the far-reaching significance of aggregation, I mean its time relations to the other events in the digestive cycle.   Once aggregated, the most active bacteria move no more ; when ingesta have attained to the stage of storage they are at the point of maximum shrinkage, and are at the same time ready for subsequent solution.   We find, in accordance with these facts, that the time elapsing between *ingestion* and *aggregation* is more nearly constant than any other period in the process of digestion, and that, on the other hand, the storing of ingesta may be as vigorous in character as it is varying in duration.   I have counted one hundred stored clusters of bacteria in one polype of *Carchesium*, and it has been mentioned above that no interval or an interval of many hours may be introduced between the end of the movement of retrogression and the onset of solution.   The possibility of such excessive accumulation is to be associated, probably, with the wide intervals which may elapse in the free state between successive periods of feeding ; and there is obvious fitness in the introduction of variation here, for the ingesta are reduced to inertness, and to their minimal size, and therefore make less demand than at any other moment on the functional activity of the animal in which they lie.

Note.—It remains for me to mention a modification of one phase in the digestive process to which reference has been made above. This modification is an extreme case of lethargy in the aggregation of particles. A specimen of *Carchesium* was mounted for observation at 12 noon, and for fifteen minutes showed vigorous ingestion of Indian ink; at the end of this time, coagulated proteid (probably mixed with some bacteria) was substituted for the pigment, and some further ingestion followed. Observation was then suspended for four hours, and at the end of that time ingesta were being formed by persistent or renewed activity; the particles which helped to make up the vacuoles of ingestion moved so vigorously, that I think they must have been, in the main, motile bacteria, and, watching for the act of aggregation, I saw that five, six, seven, even nine seconds elapsed after the onset of centripetal movement before there was complete quiescence. A homogeneous rim or shell of colourless substance seemed to encircle the particles which moved freely within it until such time as the stilling force was felt even to the centre of the mass.

This variation illustrates an extreme case of initial localization of the retractile substance; the exceptional factors which are obvious here and may help to give the phenomenon its exceptional characters are, preceding secretion of some magnitude on the one hand—on the other, vigorous movements of ingesta; it may be then that the formation or outpouring of the forerunners of the viscid matter was languid, or that, this being so, the actively motile particles determined, in some fashion, its localized formation; on the next day the same polypes showed instantaneous *aggregation*, so that the whole condition was only temporary.

Summary.

1. *Carchesium polypinum* offers in many ways a particularly good field for the study of some of the processes involved in protozoan digestion; ingestion is often eager, digestion may be rapid, and the especially transparent cell substance which characterizes this animal allows the observation of both.

2. One striking feature of any specimen examined after the administration of abundant nutriment is the presence of numerous spherical masses of food; these may number one hundred in one polype of *Carchesium*, they show remarkable solidarity, and, on the whole, uniform size, and as the solid particles ingested are invariably minute, the formation of the relatively large succeeding ingesta is a matter of some interest.

3. Continued watching shows that each spherical food mass springs from one vacuole of ingestion, and in the following fashion : the vacuole discharged internally from the œsophagus by some obscure impulse, and made up of water, and (as the case may be) amorphous matter, motile particles or inert particles, passes towards the base of the animal. It pauses internally at the curve made by the nucleus in this region, and without further locomotion, or after slight rotatory movement the finely divided solids which it contains undergo a sudden and striking rearrangement. All the granules present are shifted centripetally, all individual movement is stilled. A composite solid, lying in clear fluid surroundings, represents the scattered particles of a moment ago.

3A. To this centripetal shifting the term *aggregation* may be applied conveniently. It is demonstrable most effectively in vacuoles which are made up of much fluid, and but little finely divided solid

matter; it is masked sometimes by the number or size of the particles involved. Further, when the ingested matter has a certain definitely complex composition (and there is some evidence for the view that a soluble food stuff must be present), there is a more pronounced modification of action; two centripetal movements of matter may be distinguished—the first, a quick rearrangement by which most of the discrete particles present are gathered to form a central "nucleus"—the second, a prolonged retraction of matter separated out symmetrically or asymmetrically from the clear vacuolar fluid, and fitting finally like a shell or cap over the mass first laid down.

3B. From the point of time at which the centripetal gathering of substance takes place the fluid of the digestive vacuole begins to show an acid reaction.

4. The spherical food masses thus welded from scattered fragments then journey through the substance of *Carchesium* in a fairly constant fashion, but for a variable time; they are stored occasionally for some hours, while at times the beginning of digestion follows at once on the act of aggregation. All the ingesta present are not, of necessity, digested synchronously, but this may be regarded as invariable—that the storage of matter for any length of time before digestion is accompanied by loss of fluid surroundings, and that solution is effected in a fluid medium. Stored ingesta are constantly dense and shrunken; solution implies swelling, transparency, the persistence or re-formation of a well-marked vacuole.

5. Digestion may take place at any point throughout a relatively large part of the central substance of *Carchesium*, but the region from which insoluble matter is rejected, is, like the place of ingestion, definite. A vacuole of ingestion passes into the protoplasm from the extreme internal point of the œsophagus; effete matter is passed into the pharynx at the junction of its external and middle thirds from some spot in a ridge running transversely to the long axis of the polype in that region.

6. The ingestion of matter by *Carchesium* is indiscriminate when the particles concerned are sufficiently small, and nutritious and innutritious substances exhibit alike the striking centripetal clustering which has been described; the intra-cellular sojourn of innutritious bodies is curtailed however, the vacuoles in which they lie at first tend to disappear quickly, and there is but rarely that re-formation of fluid which is so nearly concerned in the solution of true food-stuffs.

EXPLANATION OF PLATE.

Fig. 1. Plan of *Carchesium*, to show the typical path of nutritive ingesta; purely diagrammatic. The red line marks the course from which (for the sake of clearness) some possible complications are omitted. Arrows indicate the direction of movement; the points at which striking actions occur are numbered in order.

    1. Ingestion.

    2. Quiescence.

2a. Aggregation.

3. Solution.

4. Ejection.

These points must be regarded only as the centres of areas which bear respectively the same relation to the process of digestion.   I have tried to denote this by variations in shading, which, however, must be regarded as indicating the position of the areas, rather than as defining their extent.

Area of progressive movement—shaded in rings.

Area of quiescence—shaded in crosses.

Area of retrogressive movement—shaded in dots.

Area of solution (ingesta linger here during digestion)—shaded in transverse lines.

Area of discharge (ingesta gather here before ejection)—shaded in transverse and longitudinal lines.

In all the figures, *nuc.* = nucleus.

$ph.$ = pharynx.

œs. = gullet.

Arrows indicate the movement of vacuoles, or of substances in a vacuole.

The ingesta are often arranged diagrammatically to avoid overlapping, and, in figures 3, 4, 5, and 7, are drawn with a camera lucida under Oc. 3, Obj. F. Zeiss, and afterwards reduced.   The figures are all taken from *Carchesium polypinum,* but I have attempted no reproduction of the structural details of the animal, nor of such specialized portions of its substance as the peristome and the ciliary wreath.   The outline of the polypes is drawn to scale in order that the localization of the ingesta and their size may be realized.

Fig. 2. A.—Ingestion of Indian ink suspended in water.

To show (1) usual shape of vacuole of ingestion (*ing.*).

(2) change produced by primary aggregation (*K*).

(3) ejection of a mass of Indian ink, from which outside grains have been freed (1).

(4) ejection of a mass entirely coherent, as after *aggregation* (1*a*).

B.—Concomitant ingestion of Indian ink and precipitated white of egg

ing., vacuole of ingestion.

K, aggregation.

a, b, c, d, secretion of fluid by means of which the coagulated proteid is digested.   The undigested grains of India ink remain, and in

(*d*) approach the area of discharge.

C.—Ingestion of coagulated proteid.

> *ing.*, vacuole of ingestion; proteid particles lie scattered throughout the vacuole.
>
> *s*, stage of storage.
>
> *d*, one proteid mass reduced in size by subsequent digestion.

Fig. 3. To show digestion of coagulated proteid in *Carchesium polypinum.*

> *k*, aggregation has just taken place.
>
> *a*, stage of storage.
>
> *b*, secretion of digestive fluid; increased transparency of dissolving mass.
>
> *c, d*, reduction under solvent action of fluid.
>
> *e*, undigested remnant approaches the area of discharge.

Fig. 4. To show digestion of a solid mass, probably of bacteria, which had been for some hours in the stage of storage.

> *s*, stage of storage; observation began at 11.45 A.M.
>
> *a, a'*, two ingesta sketched at 12 noon.
>
> *b*, one of these ingesta. Sketched at 12.10 P.M.; shows slight increase in transparency, and swelling.
>
> *c*, 12.18 P.M. Reduction in size begins to be marked.
>
> *d*, 12.45 P.M. Digestion has produced far-reaching change; the undigested residue still forms a coherent mass, now with shadowy ill-defined edges.

Fig. 5. To show modifications of the typical digestive act.

> *ing*, vacuole of ingestion, containing stale white of egg and Indian ink.
>
> *K*, primary aggregation; nearly all the grains of pigment have shared in the centripetal movement, but the vacuole left outside is not clearly fluid.
>
> k_1, second phase of double aggregation; a cap-like mass of substance fits over the first pigmented accumulation; clear fluid lies outside.
>
> *s*, the same mass after secondary shrinking; storage stage.
>
> *a*, later; digestion has begun, and some of the grains of pigment are set free into the fluid vacuole.

> *l, mu, ir*, from other experiments.
>
> *ir*, to show distorted aggregation, produced sometimes by the ingestion of solid particles with a viscid nutritive solution.
>
> *mu*, ingestion of Indian ink with some mucilaginous matter which was visible obscurely from the moment of enclosure.

Fig. 6. Ingesta of *Carchesium polypinum.*

> *a, a_1*, to illustrate interrupted aggregation; in *a* the terminal grains of Indian ink are still in movement; in a_1 the aggregation is com-

plete, but the grains gathered in last are not yet distinguishable from the rest.

b, vacuole of ingestion. *Aggregation* is just complete ; slender viscous threads join the central mass of proteid to the polar boundaries of the vacuole.

c, ingesta lying in a common vacuole ; the result of fusion of vacuoles during *retrogression*.

d, similar fusion of vacuoles towards the end of the stage of solution.

e, ejected mass of Indian ink ; there is a central, solid nucleus and some (?) mucilaginous basis unites the outlying granules.

Fig. 7. Ingesta of *Carchesium polypinum*.

a, *a₁*, to show extensive centripetal movement of two fat globules enclosed in a very fluid vacuole of ingestion. Arrows mark the direction of movement.

b, carmine and bacteria drawn after *aggregation ;* movement has been slight, and the composite mass is bulky still.

b₁ (the same mass drawn after two minutes) ; shrinking has gone further, the fluid of the vacuole is separated more clearly.

c, ingestion of milk ; the fat globules are held together by some almost invisible basis.

Fig. 8. Ingesta of *Carchesium polypinum*, illustrating the phenomenon of double aggregation.

a, vacuole of ingestion.

b, after primary *aggregation ;* the first solid matter separated out leaves the vacuolar contents slightly opaque.

c, the second slow movement begins.

d, the second movement is over, and a double mass lies in clear fluid.

a. In this sequence the second *aggregation* involves a movement of *granular* matter.

b, after primary *aggregation*.

c, after the second movement ; clear fluid is separated out.

Fig. 9. Adapted from R. Greef.

Diagrammatic representation of the alimentary canal in one of the *Vorticellidæ ;* to contrast the nomenclature of Greef with that of Lachmann.
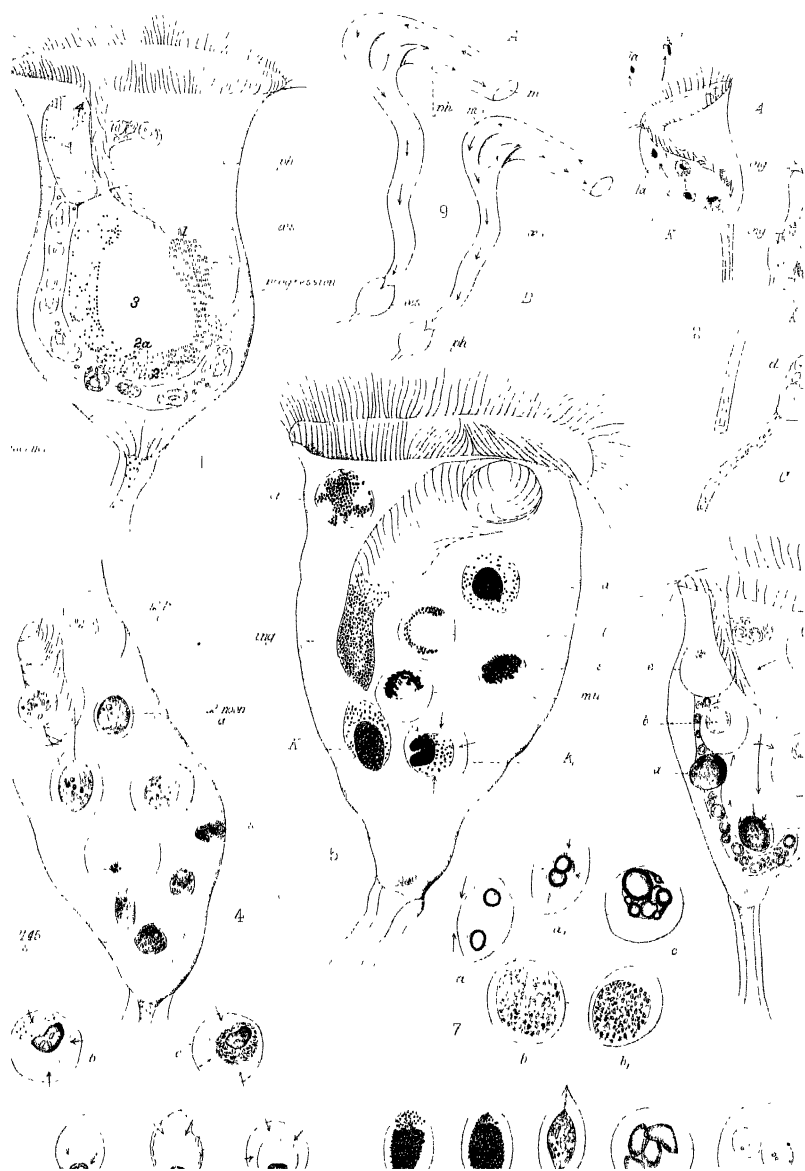
X. *Researches on the Germination of the Pollen Grain and the Nutrition of the Pollen Tube.*

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Communicated by W. T. Thiselton Dyer, *F.R.S., C.M.G., C.I.E.*

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That the deposition of the pollen grain upon the stigma is followed by a process of true germination was established by Van Tieghem as long ago as 1871,* who pointed out the similarity of its behaviour to that of the spores of Lycopodiaceæ and of many ferns, and indicated that the pollen tube may be compared to the prothallium proceeding therefrom, especially in the cases of those prothalli which contain no chlorophyll. Its growth is prepared for by the deposition in the grain of several forms of reserve material, chiefly of carbohydrate nature. During the process of germination these reserve materials have been found to change, indicating an active metabolism, while at the same time active respiration goes on, as shown later by Mangin.† Not only do we find reserve materials deposited in the pollen grain, but further, we can identify a similar store in the tissues of the style, especially when that organ is a long one, and the pollen tube has consequently some distance to travel before reaching the ovules.

In most cases of the storage of reserve materials in plants we find evidence of the utilization of such stores by the presence and activity of enzymes. Thus in seeds, Gorup-Besanez,‡ Guignard,§ Brown and Morris,‖ and others, have proved their presence ; in tubers, Baranetzki¶ found them in the potato ; in leaves, Brown and

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* Van Tieghem, " Recherches physiologiques sur la régétation libre du pollen, &c.," ' Ann. des Sc. Nat., Bot.,' 5ᵉ série, vol. 12, 1871.

† Mangin, " Recherches sur le pollen," ' Bull. Soc. Bot. de France,' vol. 33, 1886.

‡ Gorup-Besanez, 'Deutsch. Chem. Gesell. Ber.,' 1874.

§ Guignard, ' Journal de Botanique,' 1890, p. 385, *et seq.*

‖ Brown and Morris, " Researches on the Germination of some of the Gramineæ," ' Journ. Chem. Soc.,' 57, 1890.

¶ Baranetzki, ' Die stärkeumbildenden Fermente,' 1878.

MORRIS,[*] and VINES[†] have demonstrated their existence, and their relation to the temporary deposits of starch in the chlorophyll grains. The writer[‡] has shown various enzymes to exist in seeds and tubers, transforming for the nutrition of the plant, proteids, fats, and carbohydrates.

Pollen being the seat of a germinative process, physiologically comparable to those already mentioned, the probability of the activity of enzymes therein, is at once apparent. Nor is evidence wanting that such bodies exist there; VAN TIEGHEM[§] has found that when the pollen of Narcissus, Crocus, and some other species, is cultivated in 10 per cent. solution of cane sugar, in no very long time after the sowing some of the sugar becomes inverted. The result is brought about when the grains are allowed to grow freely, or when such growth is prevented by the presence of chloroform in the culture fluid.

STRASBURGER[‖] also points out that when pollen grains are allowed to grow in the presence of a very thin starch paste, a transformation of the starch into sugar can gradually be noted, indicating the presence of diastase.

The probability of certain pollen grains containing a cytolytic enzyme has been noted by other observers. Thus it has been shown that in the progress of the pollen tube of grasses through the tissue of the style it often passes between the cells, instead of through them, burrowing thus through the middle lamella. STRASBURGER[¶] notes similar behaviour of the tube in several genera of Dicotyledons, especially certain of the Caryophyllaceæ and the Malvaceæ.

In all these cases, however, the action of the living pollen grain only has been observed. The question of an enzyme capable of being extracted by appropriate solvents, and of acting while in such solution, still remains open to investigation. In a paper which the writer communicated to the British Association at Cardiff, in 1891,[**] the existence of diastase in such a condition and the possibility of extracting it, were dealt with. Since that date more extended experiments have been carried out, which form the subject of the present paper.

From the work already quoted above, the enzymes which were to be expected in pollen grains appeared to be diastase, invertase, and a cytolyst. The presumption in favour of the latter was not so strong as that in favour of the two first named,

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* BROWN and MORRIS, "A Contribution to the Chemistry and Physiology of Foliage Leaves," 'Journ. Chem. Soc.,' May, 1893.

† VINES, 'Brit. Assoc. Reports,' Cardiff, 1891.

‡ GREEN, "On the Changes in the Proteids in the Seed, &c.," 'Phil. Trans.,' vol. 178, 1887, B. *Ibid.*, "On the Germination of the Seed of the Castor-oil Plant," 'Proc. Roy. Soc.,' vol. 48, p. 370. *Ibid.*, "On the Germination of the Tubers of the Jerusalem Artichoke," 'Ann. of Botany,' February, 1888.

§ VAN TIEGHEM, "Inversion du Sucre de Canne par le pollen," 'Bull. Soc. Bot. de France,' vol. 33, 1886.

‖ STRASBURGER, "Ueber fremdartige Bestäubung," 'Jahrb. f. wiss. Bot.,' vol. 17, p. 94.

¶ "Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen," 1884.

** 'Brit. Assoc. Reports,' Cardiff, 1891, p. 696.

though it was supported by the discovery of such an enzyme by MARSHALL WARD in the hyphæ of a species of Botrytis examined by him in 1888.* These hyphæ were found to perforate cell walls in much the same way as pollen tubes make their way through the tissue of the style and ovary, and they yielded on appropriate treatment a fluid which softened and dissolved cellulose, just as the extracts of the scutellum of Barley grains prepared by BROWN and MORRIS.† The appearance and mode of growth of the pollen tube simulate very closely the phenomena observed by MARSHALL WARD in the development of the mycelium of the Botrytis.

The method adopted in investigating the existence of isolable enzymes in pollen was the following. Flowers were selected whose stamens were just beginning to dehisce and the pollen extracted from them by careful dissection. When a large quantity of pollen was required, such stamens were taken out and allowed to dry at the ordinary laboratory temperature in watch glasses ; they slowly opened in drying and were then well shaken and sifted through fine muslin. The pollen so collected was ground up in a glass or agate mortar, till microscopic examination showed most of the grains disintegrated. In some cases the powder was then mixed with thin starch paste (1 per cent.), and the effect observed. In others, the powder was suspended in either glycerine or 5 per cent. solution of NaCl, to which ·2 per cent. of KCy was added as an antiseptic. In yet other cases, a few drops of either chloroform or oil of cinnamon were used to keep away bacteria, the latter essence having been shown by CADEAC and MEUNIER‡ to be a strong bactericide. The extracts were allowed to stand for a few hours and then filtered, and the resulting filtrate used to act on starch paste, or solution of cane sugar. Sections of delicate cellular tissue were used to examine for the cytolyst, being put in a small quantity of the extract in watch-glasses. The digestions were carried out either at the ordinary laboratory temperature, or at 38° C. in an incubator, controls, in which the extract had been boiled for several minutes, being carefully kept in each experiment. In many cases it was found that the pollen extracts themselves had the power of reducing FEHLING's fluid. When this was the case, a further control was prepared, consisting of the extract diluted with water instead of either cane sugar solution or starch paste, the proportions being carefully adjusted in each set, so that they might be strictly comparable. After a sufficient time had been given for action, the quantity of reducing sugar formed in each digestion tube was determined by the method of boiling with excess of FEHLING's fluid, filtering, combustion, and weighing the resulting CuO. When chloroform or oil of cinnamon were used as antiseptics, they were removed by boiling the solution till they had evaporated, before titrating with the FEHLING's fluid.

* 'Annals of Botany,' II., 319.

† "Researches on the Germination of some of the Gramineæ," 'Journal of the Chemical Society,' June, 1890, 458.

‡ 'Ann. de l'Inst. Pasteur,' vol. 3, 1889, p. 317.

In the case of diastatic action, the effect of adding iodine from time to time to a portion of the digestion was also noted.

Details of some of the experiments in each case are appended.

I. *Diastase.*

Lilium candidum.—1. Ground pollen used without extraction—A quantity of the ground pollen, the contents of two anthers, was mixed with 10 cub. centims. of thin starch paste of 1 per cent. strength, 5 cub. centims. boiled and cooled, 5 cub. centims. left as prepared.

After 46 hours the unboiled tube gave a purple colour with iodine, the boiled one a blue. After 24 hours longer action the unboiled tube gave no colour with iodine, while the control became blue. Testing now with FEHLING's fluid, the unboiled tube gave a copious reduction, the control gave none.

2. Glycerine extract prepared from pollen from several stamens, as described above. When filtered, 5 cub. centims. were boiled, and 5 cub. centims. taken as prepared. Each was mixed with 5 cub. centims. starch paste and exposed at the temperature of the laboratory. At intervals, the two were tested with iodine, usually ten drops being taken from each. The results are expressed in the following table.

Time of digestion.	Unboiled e	Boiled extract.
26 hours	Purple-]	
2 days	Purple	
3　　,,	Red-bro	
6　　,,	Colourl‹	

The contents of the tubes were then boiled with FEHLING's fluid, when the unboiled gave a copious reduction and the boiled one a faint trace.

Though the action was carried on for several days, no bacteria appeared in either tube. The filtrate, as prepared was quite clear and free from any *débris* of pollen.

Helianthus.—Pollen very small. 1. Mixed at once with 1 per cent. starch paste, and half the mixture boiled. Digested both at 18°C. and at intervals tested a few drops with iodine.

Time.	Unboiled.	Boiled.
2 days.	Red-purple	Blue
3　　,,	Colourless	,,

Boiling both these digestions separately with FEHLING's fluid, the unboiled gave a copious reduction, the boiled one scarcely a trace.

2. Glycerine extract of ground grains prepared as in case of Lily.

Time of action.	Unboiled.	Boiled.
1 day	ddish-purple	Blue
2 days	d-brown	,,

The action was stopped at this point, and each tube boiled with FEHLING's fluid. The unboiled reduced it copiously, the boiled one gave hardly a trace of Cu_2O.

Corylus avellana.—Extract made by steeping ground pollen in 5 per cent. NaCl + ·2 per cent. KCy solution for twenty-three hours and filtering. In this case further controls were prepared, the tubes being mixed as follows :—

A. 5 cub. centim. extract + 5 cub. centims. 1 per cent. starch paste.
B. 5   ,,   ,,   boiled + 5 cub. centims. 1 per cent. starch paste.
C. 5   ,,   extracting fluid + 5 cub. centims. 1 per cent. starch paste.
D. 5   ,,   water + 5 cub. centims. 1 per cent. starch paste.

These were left to digest at 20° C. in the laboratory, with the following results when samples were treated with iodine.

Time of digestion.	A.	B.	C.	D.
19 hours	Red-purple	Blue	Blue	Blue
44 ,,	Red-brown	,,	,,	,,

On boiling the remainder of each with FEHLING's fluid, at the end of the 44 hours, A reduced it copiously, the others not at all.

Comparing this experiment with those previously narrated, the advantage of the salt solution over glycerine as a solvent is evident.

Lilium pardalinum.—A more careful quantitative experiment made with the pollen of this species may be quoted here, though it was made with another object which will be referred to later. ·3 grm. of pollen was weighed out, carefully ground up and mixed with 60 cub. centims. of starch paste (1 per cent.). After agitation to ensure an equal diffusion of the *débris* with the starch, it was rapidly divided into two, and half of it heated for fifteen minutes in a water bath to 98° C. The two, labelled P and P*b*, were then put for three days in an incubator at 38° C. At the end of that time P was perfectly limpid and clear, while P*b* was apparently unchanged. No bacteria had developed. They were both then passed through filters to remove the *débris* of the pollen, which had settled to the bottom of the beakers, and the filtrates boiled with excess of FEHLING's fluid. Both gave a reduction, that of the control being less than the other, and no doubt due to a reducing sugar which other experiments

had shown to be present in the pollen of this species, as will be described later. The resulting precipitate was thrown in each case on to a filter, washed, dried, and after combustion in a crucible, weighed as CuO. The results were as under :—

	P.	Pb.
Gross weight of crucible, ash of filter, and CuO	1·357 grm.	1·282 grm.
Weight of crucible and ash	1·246 ,,	1·246 ,,
Weight of CuO	·111 ,,	·036 ,,

There was a quantity of reducing sugar in the grains of pollen represented by ·036 grm. CuO. Deducting this from the CuO found in P., we have ·075 grm. of CuO, due to the sugar formed by the diastase in half the ·3 grm. pollen used.

These experiments show that diastase exists in pollen, and that it can be extracted as readily from the grains as from the cells of other parts of the plant.

Besides the species already mentioned, experiments showed its presence in the pollen of Gladiolus, Anemone, Antirrhinum, Tropæolum, Pelargonium, Crocus, Brownea, Helleborus, Alnus, Tulipa, and Clivia; also in that of Zamia after germination begins. The action on starch grains suggests that it belongs to the translocation variety of Brown and Morris,* as it dissolves the starch grains without corrosion. More on this point will, however, be described in connection with experiments made on the pollen tube and its contents.

II. *Invertase.*

The first experiments made with a view to the identification of this ferment were qualitative only.

Eucharis grandiflora.—Pollen was collected from several anthers just commencing to dehisce, and was ground up in an agate mortar, and mixed with 10 cub. centims. of solution of 5 per cent. NaCl and ·2 per cent. KCy. After standing 20 hours it was filtered till clear; 2 cub. centims. of this extract were then mixed with 10 cub. centims. of a weak solution of cane sugar, and a similar control was prepared with 2 cub. centims. of the extract boiled.

The two were tested at intervals by boiling 1 cub. centim. of each with 1 cub. centim. of Fehling's fluid diluted with 4 cub. centims. of water. Results were as under :—

* *Op. cit.* Also Green on "Vegetable Ferments," 'Ann. of Bot.,' vol. 7, p. 86.—March, 1893.

Time.	Unboiled.	Boiled.
After 19 hours . .	Trace of reduction	No reduction
„ 43 „ . .	Liquid turned yellow, but gave no precipitate	Faint greenish tint replaced the pure blue
„ 6 days . .	Copious reduction and precipitate of Cu_2O	No further reduction

The pollen used in this case was only the contents of three or four small anthers, but the result shows that a workable quantity of invertase can be extracted from even so little.

Narcissus papyraceus albus.—The pollen, after grinding up, was extracted in this case by 10 cub. centims. dilute glycerine, to which ·5 per cent. of asparagin had been added, according to the suggestion of EFFRONT,[*] the amide having the property of accelerating the action of any enzyme present.

After extraction the residue was filtered off and suspended in another 10 cub. centims. of the same mixture, with a view to ascertaining whether all the enzyme could be extracted by one exposure to the fluid. The extracts were labelled A and B respectively.

5 cub. centims. of each was then boiled, and digestion tubes were prepared as under :—

A. 2 cub. centims. extract A + 2 cub. centims. solution of cane sugar ;
B. Boiled control ;
C. 2 cub. centims. extract B + 2 cub. centims. solution of cane sugar ;
D. Boiled control ;
E. 2 cub. centims. glycerine mixture + 2 cub. centims. solution of cane sugar, without either extract.

All were placed in the incubator at a temperature of 38° C.

After 24 hours a small sample of each was boiled with FEHLING's solution. A gave a strong red reduction ; C a yellowish-red one ; while the controls all alike showed a faint greenish tinge, presumably due to a trace of inverted sugar in the cane sugar used.

After a further 24 hours the differences noted were intensified, showing a progressing inversion in A and C. The controls remained as before, all showing a very slight trace of reduction.

The pollen was thus shown to contain invertase, and to yield it up only incompletely to extraction.

Some quantitative experiments were subsequently made upon two other species.

Narcissus pseudo Narcissus.—A quantity of pollen, weighing ·4 grm., was ground

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[*] EFFRONT. "Sur les Conditions Chimiques de l'Action des Diastases," 'Comptes Rendus,' vol. 115, p. 1324.—December 26, 1892.

up in an agate mortar, and mixed with 100 cub. centims. of salt mixture (5 per cent. NaCl + ·2 per cent. KCy), and allowed to extract for several days. The mixture was then filtered, and the filtrate examined microscopically, and found to be free from bacteria. As invertase had been demonstrated to exist in an allied species, a boiled control was not employed in this experiment.

Two tubes were prepared, one containing the extract + 100 cub. centims. of cane-sugar solution, and the other 100 cub. centims. of the same salt mixture as that with which the pollen had been extracted, added to the same quantity of sugar solution. After three days' action, the two were boiled with excess of FEHLING's solution, and the resulting precipitate was filtered off, washed, and subjected to combustion in a platinum crucible. The quantity of CuO from the one was ·038 grm., and from the other ·021 grm., giving ·017 grm. CuO due to invert sugar, produced by the action of the invertase on the sucrose.

Narcissus poeticus.—·1 grm. of pollen was taken and extracted in 15 cub. centims. chloroform water, without bruising, for two days. Then it was filtered, and the filtrate added to 25 cub. centims. of 10 per cent. solution of cane sugar. A control was prepared, consisting of 15 cub. centims. chloroform water and 25 cub. centims. of the same sugar solution. A few drops of oil of cinnamon were added to each as a further antiseptic,* and the two were digested at the ordinary laboratory temperature for four days. Then, after boiling for some time till the chloroform and oil of cinnamon were removed, they were again boiled with excess of FEHLING's fluid, and the oxide filtered off, washed, heated in platinum crucible to redness till the weight was constant, and weighed. The control gave ·0098 grm., the pollen extract gave ·0978, or nearly ten times as much.

Besides these pollens, invertase was found in that of *Helleborus,* of *Richardia* (the so-called Arum-lily), of *Lilium pardalinum,* and of *Zamia skinneri.*

Of other pollens examined for the two enzymes, diastase was found to be absent from Lupinus, Lathyrus, Eucharis, Richardia, and Narcissus; invertase was not found in Alnus and Clivia.

A few experiments were made with a view to determining the existence of a cytolyst and a proteolyst, but in no case could either be found.

The enzymes present in resting pollen grains are, therefore, chiefly diastase and invertase, but their distribution is irregular, some containing one, some the other, and some both. Where diastase occurs, it is the form described by BROWN and MORRIS as the " translocation" variety. This is apparently the form indicated in STRASBURGER's experiments already referred to.

The enzymes are with difficulty completely extracted by solvents, even several days' action of the various extracting fluids leaving some behind in the residue. Of the solvents used, 5 per cent. NaCl solution is the most effectual.

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* CADÉAC, *op. cit.*

Changes in the Quantity of Enzyme during the Germination of the Pollen Grain and the Growth of the Pollen Tube.

Many experiments were made with various pollens to ascertain which species would germinate most freely, and in what culture fluids they could most easily be made to put out pollen tubes. Eventually, various species of *Narcissus* and of *Lilium* were selected as yielding invertase and diastase respectively. The pollen of these genera was found to germinate in water, and in various strengths of cane-sugar solution. Some experiments made with the pollen of *Zamia skinneri* also yielded instructive results.

In making these experiments, a quantity of pollen was collected from several hundreds of anthers, and equal weighed quantities were cultivated on glass plates under bell jars over water. As it was impossible for them to grow in the presence of antiseptics, the cultures were carefully watched and examined at short intervals to guard, as far as possible, against the danger of ruining the experiment by the introduction of micro-organisms. In many cases the tubes attained a good degree of development in a few hours, some species of Lily putting them out in two hours or less. In other cases, the cultures proceeded for one or two days in safety. After the culture was made, the germinating grains and their tubes were digested under various conditions with either cane sugar or 1 per cent. starch paste, further germination being inhibited by addition of antiseptics, usually ·2 per cent. of potassic cyanide. Controls with ungerminated pollen, or extracts of it, were kept side by side with the others. In some cases, the cultures were dried on the plates at a low temperature (40° C.), and the dried residue collected and ground up in the agate mortar, the controls in each experiment being treated in the same manner as the cultures.

The most striking experiment with invertase was made on the pollen of *Narcissus poeticus*. The pollen was collected from 906 anthers, a quantity weighing ·3 grm. being yielded by this number. This was divided into three parcels of ·1 grm. each. One parcel (A) was steeped at once in 10 cub. centims. chloroform water; another (B) was set to germinate in water on a glass plate; and the third (C) similarly in cane-sugar solution (15 per cent.). When germination was well advanced the cultures were carefully washed from the plates, and all made up to 15 cub. centims. with chloroform water. They were then all filtered, mixed with 25 cub. centims. of 10 per cent. cane-sugar solution, and allowed to digest for 93 hours at the ordinary laboratory temperature.

At the expiration of this time, after removal of the chloroform by boiling for some time, 20 cub. centims. of each were again boiled with excess of FEHLING's solution, and the copper oxide filtered off, washed and weighed, after combustion, in a platinum crucible.

A yielded ·1 grm. CuO; B, ·24 grm.; and C, ·65 grm.; a blank experiment showing ·01 grm.

Calculating the sugar produced in each case in 100 cub. centims. of the digestion

of the Lily has a very strongly thickened coat, impregnated with a considerable quantity of a resinous colouring matter, rendering extraction a matter of some difficulty. An experiment was consequently made to ascertain whether extraction could be complete by the use of a solvent, or whether, as in so many cases, particularly of leaves,[*] the residue of the grains after extraction retained much of the diastase. Two parcels of pollen of *L. pardalinum* were taken, each weighing ·2 grm. One was put to germinate in water, and the other steeped at once in water containing ·2 per cent. of potassic cyanide to inhibit this process. After the tubes had obtained a fair length, the culture was filtered and the filter washed with water, the washings being added to the filtrate. The grains, with their tubes, were then extracted with the usual salt mixture for 48 hours, when the extract was filtered off. The residue was then suspended in a further quantity of the extracting fluid. The other parcel was treated similarly, so that there were prepared from each a filtrate from the culture, a salt extract of the grains, and a residue suspended in fluid. Each of the six was made up to 25 cub. centims., and the KCy adjusted that each should contain ·2 per cent. of the antiseptic. The two sets were labelled G and H respectively, and each 25 cub. centims. was mixed with 30 cub. centims. of 1 per cent. starch paste. Digestion was carried out for three days at 18° C., its progress being noted by testing a few drops with iodine at regular intervals, and finally titrating with FEHLING's fluid. The first outcome of the experiment was that the total diastase was increased by about 50 per cent. in the germinated pollen. There was a good deal of difference in the distribution of the diastase in the several digestions of the two sets. Of the total amount found in the G set, 45 per cent. was in the filtrate, 29 per cent. in the extract, and only 26 per cent. in the residue; while in the H set, with a smaller total quantity, 23 per cent. was in the filtrate, 13 per cent. in the extract, and as much as 64 per cent. in the residue. This shows how difficult it is to extract the enzyme from the thick-walled pollen grain, and how relatively easy to obtain it from the thinner-walled pollen tube. The total result, however, shows that the increase observed in the whole experiment is a real one, and not a question of incomplete extraction.

One experiment made upon this pollen appears to indicate that the increase noted above is not an immediate one, but that it is preceded by a diminution during the early stages of the growth of the tube. This is a different result from that arrived at in the case of the invertase, and shows that the process is not exactly alike in all cases. Too much stress should not be laid upon it, though the results are rather striking. It was made with the last sample of the pollen of *L. pardalinum* which would germinate, and for want of fresh material it could not be repeated. The probable explanation of the result will be dealt with later, when discussing the general question of the formation of the pollen tube. In conducting the experiment two parcels of the pollen, each weighing ·1 grm., were taken; one was ground up at once

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[*] *Cf.* BROWN and MORRIS, "On the Chemistry and Physiology of Foliage Leaves," 'Journal of the Chem. Soc.,' May, 1893, p. 634. Also VINES, 'Annals of Bot.,' 1891, p. 409.

and steeped in water, the other was germinated for seven hours, till fair tubes had made their appearance.   The culture was then removed from the plate, and the grains and tubes ground up as in the case of the other parcel.   Without filtering, each was mixed with 20 cub. centims. of 1 per cent. starch paste, and put in the incubator at 38° C.   At the end of 18½ hours both were filtered rapidly, the filtrates boiled with excess of FEHLING'S solution, and the resulting precipitate treated as usual.   The final weights of CuO were—

From the digestion with germinated pollen ·036 grm., corresponding to ·027 grm. maltose.

From the digestion with ungerminated pollen ·0595 grm., corresponding to 044 ·grm. maltose.

(1 grm. of maltose reduces 1·345 grm. of CuO.)

The course of action in the pollen of *Lilium pardalinum* appears, therefore, to be that during the first few hours of germination, there is a diminution of the quantity of diastase, followed by a recovery and subsequent increase.

Some further experiments with reference to the existence of diastase were carried out on the pollen of *Zamia skinneri*, one of the Cycads.   The pollen grains of this plant differ from those of the Lily in not containing starch as a reserve material, though when their tubes are growing in a suitable environment, starch soon makes its appearance in them.   The pollen grains of *Zamia* are roundish to oval in shape, with a crease-like mark down their longest diameter.   They will not germinate in water, but will do so fairly readily when sown upon pieces of boiled or raw pear or apple pulp. Less freely they may be cultivated in the expressed and filtered juice of either of these fruits.   As said above, they contain no starch.   Examination was made by mounting them in a strong solution of chloral hydrate, to which a little alcoholic tincture of iodine had been added.   This reagent slightly swells the grains, and at the same time renders them extremely transparent, while the iodine colours any starch that may be there.

Experiments failed to show any sufficient evidence of diastase in the resting grain, though it was sought for carefully, as starch soon appeared when the germination began.

Several experiments made with apple juice and its various constituents taken separately, soon showed that the question of the growth of the tube mainly turned on the question of the absorption of carbo-hydrate material, and that the vegetable acid of the juice is not essential, though it is possibly advantageous.   The process of germination was very slow, so slow indeed that usually the cultures were spoiled by the growth of moulds, the mycelia of which could be seen to be infesting the cone. In all cases, however, the output of a pollen tube was preceded by the appearance of starch in the grains, and it was soon possible to detect the commencement of germination by this occurrence, which generally was noticeable about twenty-four hours after sowing the pollen.

To determine whether germination was accompanied by a development of diastase, which would probably mean the same thing as an increase of the original quantity in the case of the Lily, three equal parcels of pollen were taken. One was steeped in water, and one in apple juice, while the third was used dry. After two days the grains soaked in juice were swollen and contained numbers of starch granules ; those steeped in water were swollen like the others, but contained no starch : their protoplasm was somewhat more granular than before. The two were then dried in the incubator at 38° C., and all three ground up separately, and extracted with ·2 per cent. KCy solution for twenty-four hours, the acidity of the residue of the juice being carefully neutralized before extracting. The extracts were filtered, and mixed with a little 1 per cent. starch paste. Care was taken especially to see that all three were exactly alike in the quantity of starch, of KCy, and of water, also that the reaction was alike in all three.

Each was then divided into two, and half of it boiled for fifteen minutes on a water bath. Action was allowed to proceed in the incubator at 38° C. for three days, when it was stopped, and the several digestions boiled with excess of FEHLING's fluid. The resulting precipitates were collected on filters of known ash, and washed with hot water. The filters were then dried and subjected to combustion in a platinum crucible till the weight was constant.

The results were as under :—

Juice culture.	Unboiled tube.	Boiled tube.
Weight of crucible, ash, and CuO	grms. 1·304	grms. 1·298
Weight of crucible, 1·244 grm. } ash ·001 „ }	1·245	1·245
Weight of CuO	·059	·053

giving ·006 grm. reduced by the maltose produced by the diastase in the germinating grains.

Neither the resting grains, nor those that had been steeped in water gave any evidence of diastatic action, there being only the merest suggestion of reduction on boiling the digestions with FEHLING's fluid.

In *Zamia*, then, as starch is produced after the absorption of sugar by the pollen grains, and before visible germination commences, there is a simultaneous formation of diastase to provide for its digestion. No such formation takes place, unless the sugar is absorbed. Either cane sugar or grape sugar will give rise to this appearance of starch. What the antecedent condition of the diastase may be, or whether it is secreted by the protoplasm when required, is a point that will be referred to later.

One more curious fact with regard to the diastase of the pollen of *L. pardalinum*

may be narrated here.   Attempting to confirm the initial diminution of diastase on the commencement of germination, as described above, the pollen was found to have lost its power of putting out tubes.   Out of a large quantity sown, very few grains even commenced growing.   It seemed desirable to investigate the diastatic power of the pollen in this condition, and to see whether any connection could be traced between the power of germinating and the activity of the enzyme.   The pollen remaining weighed ·3 grm. ; it was ground up in an agate mortar and mixed with 60 cub. centims. of starch paste (1 per cent.).   Half of it was then boiled for fifteen minutes, and the two were set side by side in the incubator at 38° C., labelled P and Pb respectively.

The digestion was continued for 22·75 hours, when both were boiled with excess of FEHLING's fluid, and the resulting precipitates collected, treated as usual, and weighed as CuO.   Deducting the small amount yielded by Pb, which was due to a little sugar in the pollen, P had formed sugar corresponding to ·075 grm. CuO, which, estimated as maltose, equals ·057 grm.

While this pollen still retained the power of germination, the experiment described on pp. 396–397 had been carried out with it, and as the diastase in the grains before germination was then determined, the two experiments may now be compared.   In the first, ·05 grm. of pollen yielded diastase which, working in the presence of excess of starch, formed ·044 grm. maltose in 18·5 hours.   In the second, ·15 grm. pollen, working under the same conditions exactly, formed ·057 grm. maltose in 22·75 hours. Reducing these two to ·05 grm. pollen, working for one hour, we get, in the first case, a formation of ·0024 grm., and, in the second, of only ·0008 grm. of maltose, showing that with the failure of power to germinate, the amount of diastase was reduced to one-third the original quantity.

Growth and Nutrition of the Pollen Tube.

The variations in the amount of enzyme obtainable from the grains at different periods of their life, taken in connection with the different contents of the grains of various species of plants, suggested that the growth and development of the pollen tube is not a uniformly simple process, but one showing a very definite relation to the environment in which each finds itself, and to the various nutritive materials occurring in the grains themselves, and in the styles of the plants to which they belong.

To examine this in some detail was the object of many experiments, of which the most important were made upon the pollen of *Narcissus, Lilium,* and *Zamia.*   All these can be made to germinate with fair success, the last named being the most refractory, and its cultivation, for the reasons already stated, being attended with most difficulty.

The grains were sown in various media, in hanging drops in closed glass chambers which could be transferred to the stage of the microscope.   The most convenient form

of chamber was that first used by Professor MARSHALL WARD. It consisted of a glass tube, in the centre of which an oval bulb was blown. This was broken above and below, and the two apertures of the fracture ground smooth. One aperture was then cemented to a glass slide, while a coverslip, on which was placed the hanging drop, was laid upon the other, the chamber being kept full of moist air by loosely plugging the ends of the tube with wetted cotton wool. The chamber and the cover-slip were luted by a little olive oil. In these tubes the cultivation proceeded satis-factorily for several days.

The grains of Narcissus grew fairly well in drops of water, but were best developed in solution of cane sugar, 15 per cent. being found to be the most favourable degree of concentration. After two or three days, the tubes attained a length of 20 or 30 times the diameter of the pollen grain. They were long narrow tubes with clear transparent walls, and had usually somewhat dilated ends, in some cases forming globular swellings, which were often larger than the grain itself. These globular ends had softer and thinner walls than the rest of the pollen tube. When, as was not infrequently the case, the end did not dilate, the walls of the tip were thicker than those of the rest of the tube. The mode of growth suggested a good deal of internal tension, accom-panied usually by a softening of the tip, much like that of the hyphæ of Botrytis, as described by Professor MARSHALL WARD.* In most cultures the globular swellings did not appear, and it is probable that they are abnormal appearances produced by mal-nutrition. In the Lily pollen tubes they never occurred, the wall there not being particularly different at the ends and along its length. The contents were always vacuolated, with an accumulation of granular matter, particularly towards the tip. The granules were very large and refringent. In external contact with the tip of the pollen tube generally a large number of these refringent granules appeared, looking as if extruded from the tube. That this was the case seems probable, for, in many instances, the two masses of granules within and without the tube seemed almost continuous, the thin wall, however, being usually visible between them. In one case an appearance was presented supporting strongly the idea of excretion from the tube ; this was in a tube of *Narcissus poeticus*, where a well-defined aperture, with regular and even edges, was visible on one side of the tip just behind the apex, and the granules could be seen streaming from it. This aperture had not the appearance of an irregular rupture, which was observed many times in swollen tubes.†.

The protoplasm surrounding the vacuolation all along the tubes was extremely granular, and the more so the more quickly the tubes had been developed. Very marked movements or currents of circulation were exhibited by the protoplasm,

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* *Loc. cit.*

† VAN TIEGHEM, in his paper on pollen already referred to, describes similar appearances. He says the tubes are often pierced at the end of the terminal swelling, sometimes at a single point, when the greater part of the plasma escapes in the form of a large drop or tear, and sometimes at many points, each then exuding a globule. *Loc. cit.*, 1871.

particularly in the tubes of the Lily. The tubes of *Zamia* developed very slowly, and the culture was always ruined by the appearance of mould before they had attained a length of more than five or six times the diameter of the pollen grain. They were chiefly noteworthy for certain peculiarities in their reserve materials.

In most of the cultures, besides the appearances described leading to the hypothesis of extrusion of granules without rupture of the tube, a large number of the tubes ruptured irregularly, often suddenly, with a violent expulsion of the finely granular protoplasmic contents. Sometimes a piece of the tube was broken off, sometimes the split was lateral. This appearance is no doubt abnormal, and due to excessive absorption of water from the liquid culture medium, which is not the environment naturally furnished. The distension of the tip alluded to is possibly due to the same cause. The granular matter extruded from these ruptures was very different to the refringent granules leaving the tube at the apex and remaining for a time in contact with it.

During this process of development and growth it is evident that the tube must receive nutriment in some form. The appearances described and the evidence already given of the excretion of ferment into the culture fluids point to a source of this nutriment in the tissue of the style. At the same time, it has been shown by DE PLANTA, MANGIN, and others, that the grain itself is a storehouse on a small scale, various grains differing in the nature of their contents, the latter, however, being almost always starch or some form of sugar.

The presence of starch in those pollen grains in which it exists can be readily demonstrated by mounting the mature grains in strong solution of chloral hydrate to which a little alcoholic tincture of iodine has been added. After a few hours the chloral hydrate renders the grains nearly transparent, while the iodine stains the starch. When treated in this way the starch grains appear usually as very minute specks embedded in the brown-stained protoplasm. Their number varies very much, some grains staining almost black from the amount present; others showing the isolated specks with great distinctness. Examining pollen of *L. pardalinum* in different stages of development it became evident that the starch begins to be deposited there early in the maturing of the anthers, and that the quantity gradually increases till the pollen grains are mature. In ripe grains some granules were seen to give not a blue, but a purplish-red colour with the iodine.

In many pollen grains the quantity of starch increased at the onset of germination when the latter took place in a nutrient fluid.* In *Zamia*, as already mentioned, no starch is present in the resting grain, but germination is always preceded by its appearance. As this secondary storage is not observable in water cultures, the inference is clear that a larger supply of nutritive material than serves for immediate requirements leads to the reinforcement of the reserves of the resting grain.

A second reserve material, not uniformly present, is dextrin, to which the purple-red

* This was noted by MANGIN in his experiments on respiration of pollen, *loc. cit.*, p. 517.

colour of certain of the granules is due. This is probably not a constant constituent, but due to enzyme action within the grain, as will appear later.

The presence of various sugars has been pointed out by many writers, particularly by DE PLANTA, who found 14 per cent. of cane sugar in the pollen of *Corylus avellana*,* and 11 per cent. in that of *Pinus*. In experiments on this group of constituents, the pollen of *Lilium pardalinum* was especially examined for the presence of cane sugar. A quantity of pollen was taken, which weighed 2·346 grms. It was carefully washed with ether till all colouring matter was removed, when its weight was 2 061 grms , showing a loss of ·285 grm , or 12·15 per cent. due to resin and colouring matter. It was then extracted with boiling absolute alcohol on a water bath, an inverted condenser being fitted to the flask. After an hour it was set aside to cool, and allowed to remain under the alcohol for some days. The latter was then decanted off, and the pollen again extracted as before with a fresh quantity of the spirit. The second extraction was followed by a third, conducted similarly. The alcoholic extracts were mixed together and evaporated to dryness on a water bath, when a sticky residue was left. This was dissolved in 36 cub. centims. of water and the solution divided into two equal quantities. Half was mixed with an appropriate quantity of pure invertase† and digested in the incubator at 36° C. for several hours, to invert any cane sugar present.

After this digestion was complete, both quantities were boiled with excess of FEHLING's solution, the resulting precipitates collected on filters of known ash, washed, dried, and incinerated in a platinum capsule till the weights were constant. These were found to be as under :—

	Inverted half	Uninverted half
Crucible + ash + CuO	1 413 grms.	1 3065 grms
Crucible + ash	1 251 ,,	1 251 ,,
CuO	0 162 ,,	055 ,,

The increase in reducing power was due to the inversion of cane sugar by the invertase. In the 36 cub. centims. of extract there was therefore cane sugar corresponding to 2 (0·162 — ·055) or ·213 grm. CuO. The invert sugar arising from the inversion of 1 gram of cane sugar reduces 2·321 grms. CuO, therefore ·213 grm. CuO corresponds to ·091 grm. cane sugar. As this quantity was found in 2·346 grms. pollen, the latter contained 3·9 per cent.

The other sugar was most likely (though not certainly) maltose, judging from the

* "Ueber die chemische Zusammensetzung des Blüthenstaubes der Haselstaude," 'Landwirth. Versuchs ,' 6ᵉ Reshe, 1884 and 1885.

† Kindly sent me by Mr. HORACE T. BROWN.

presence of starch, a little dextrin, and diastase in the pollen grain. Computing it as maltose, there would be in the total extract a quantity corresponding to a reduction of 2 (·055) or ·111 grm. CuO. 1 gram of maltose reduces 1·345 grm. CuO, therefore the extract contained ·083 grm., which equals 3·54 per cent. of the dry weight of the pollen used.

An examination was also made of the sugars present in the pollen of *Lilium tigrinum.* This pollen contained but little starch, and was very strongly impregnated with resinous sticky matter, the grains being thereby coloured a dark red-brown.

The parcel of pollen taken weighed 2·24 grms. It was repeatedly extracted with ether as before till the solvent came away colourless. Dried and weighed, it was found to have lost ·7 grm., or 31·2 per cent. It was then extracted as before several times with boiling absolute alcohol, the several extracts mixed and evaporated to dryness on the water bath. The residue was dissolved in 300 cub. centims. water and neutralized with a drop or two of weak ammonia.

The extract was now divided into three : 100 cub. centims. were boiled at once with excess of FEHLING's fluid and the CuO ascertained as before. The final weight was ·041 grm. CuO, or ·123 grm. computed for the whole quantity.

100 cub. centims. were warmed with an appropriate quantity of pure invertase in the incubator at 38° C. for several hours, and then treated as the first 100 cub. centims. This gave no evidence of inversion by increase of reducing power.

The third 100 cub. centims. were boiled for two hours with 2 per cent. H_2SO_4 in a flask provided with an inverted condenser, neutralised and titrated as the other two. The final weight of the CuO in this case was ·064 grm. or ·192 grm. computed for the whole quantity.

There being no increase brought about by the action of invertase, cane sugar was not present in this sample of pollen. The increase in the quantity boiled with acid was due to the transformation of a certain quantity of maltose to glucose ; the reduction in the unchanged extract to the presence of this maltose with probably some glucose.

The increase in reducing power is ·192 − ·123 = ·069 grm. The maltose corresponding to this is ·069 ÷ 0·976 = ·071 grm. (0·976 representing the difference between the amount of CuO reduced by 1 grm. of maltose, and the CuO reduced by the glucose to which 1 grm. of maltose gives rise when hydrolyzed). This amount of maltose computed on 2·24 grms. equals 3·17 per cent. As ·071 grm. maltose reduces ·0955 grm. CuO, and the original reduction was ·123 grm., we have left ·0275 grm., which must have been reduced by glucose. As 1 grm. glucose reduces 2·205 grm. CuO, this corresponds to ·0125 grm. of this sugar, or ·56 per cent.

VAN TIEGHEM states that the pollen of Narcissus, Crocus, Cheiranthus, and Viola, among others, does not contain cane sugar.*

* " Inversion du Sucre de Canne par le pollen," *loc. cit.*

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The sugars in different pollen grains are thus seen not to be constant ; cane sugar, maltose, and glucose all being present, separately or together, in different species.

Before dealing with the question of nutritive materials in the styles, it may be well to state what can be seen of the fate of these different reserves in the pollen grain as the tube grows. The microscope is of no use to us in the case of the sugar. No doubt a study of the various sugars present in cultures of tubes at various stages of germination would lead to valuable results as to the metabolic changes involving sugars, but such study has still to be made.

In the case of the starch, some observations made upon tubes of *Lilium pardalinum* are worth quoting. The ungerminated grains, as already said, when treated with iodine in chloral-hydrate solution, showed minute granules of starch, generally filling the cell, but mixed here and there with grains staining like dextrin. As the tube was put forth from the grain, these granules were gradually carried over with the protruding portion, and they flowed slowly down the tube as it extended. When the tube was as long as twice the diameter of the grain, they were found to be gradually changing in colour, becoming slightly purple with the iodine. The tube still elongating and the grains travelling forward, this change was more and more marked, particularly near the tip of the tube. When the latter had reached a length of 20 or 30 times the diameter of the grain, the general effect of the iodine was markedly changed. There were but few blue granules, and those in the part nearest the pollen grain. The greater part of the length of the tube was studded thickly with purple grains, and towards the tip they become nearly red. The starch was evidently in process of digestion by the diastase, ministering to the great formation of cellulose composing the wall of the tube. The granules did not change their shape and showed no corrosion, even when magnified very highly, but were gradually being digested in the usual fashion of translocation.

It has already been pointed out that there is an excretion of the enzymes from the pollen-tube into the culture fluid, indicating the same thing as probable when a grain is germinating in the normal way upon the surface of the stigma. The gradual progress of the tube down the conducting tissue of the style appears to be attended by the absorption of nourishment as it passes, for in the case of such flowers as the Lily, the length of the tube is far too great for its cellulose to be supplied from the comparatively small store of carbohydrate in the pollen grain itself. We must look, therefore, to the tissue of the style as the seat of some metabolism, having for its purpose the feeding of the pollen tube during, at any rate, the latter part of its growth.*

When examined with the microscope, the centre of the style of the Lily is seen to be hollow, and continuous with the cavity of the ovary. The cells are many layers in thickness around this cavity, the external layer being an epidermis with stomata.

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* MANGIN and VAN TIEGHEM both state that they could conduct the process of germination of pollen in a culture medium longer when the fluid contained nutritive matter than when it did not.

The central canal in the style is small, but well defined, and lined by an extremely well-marked epithelium, the cells of which are rounded or papilla-like towards the cavity, recalling very much the appearance of the epidermis of the stigma, with which they are continuous. The style has usually three fibrovascular bundles running up it, placed symmetrically.

When the sections are mounted in chloral-hydrate and iodine solution, the epithelium cells and the cells of several layers under them are found to be full or nearly full of minute starch granules, of about the same size as those found in the pollen grain. The outer layers are free from these. The path of the tube is down the canal or the cells abutting on it, the so-called "loose conducting tissue," where the starch is plentiful. A longitudinal preparation of the style, made by soaking one in the chloral-hydrate solution till it is transparent, which usually takes five or six days, shows that the distribution of the starch is still more significant. Besides being in the conducting tissue, it is plentiful in the outer soft tissue of the fibrovascular bundles, indicating a definite deposit or reserve store placed in the conducting tissue after formation in the leaves. The deposit does not extend to the stigma, but falls short just before the style opens out to form that structure, indicating that the store of reserve material here is intended for the growth of the pollen tube after it has exhausted the special store of the pollen grain.

The amount of this deposit of starch varies in different styles.

Besides starch the tissue of the gynæcium evidently may be expected to contain sugar, even if we only consider the sticky nature of most stigmas. An investigation of the nature and amount of this sugar was made on two species of *Lilium*.

L. tigrinum.—165 styles of various ages were collected. They were rapidly washed in water to remove adherent sugar from the stigmas, dried on blotting paper, and weighed while turgid, being found to weigh 37·73 grms. They were then dried, first at moderate temperature, and then at 100° C., and, after cooling, weighed again, being then 3·403 grms. They were ground up in a mortar and extracted repeatedly with boiling absolute alcohol, as in the case of the pollen estimation already detailed on p. 402. The final residue from evaporation of the alcoholic extracts, consisting of the sugars of the styles, was dissolved in 300 cub. centims. of water; 100 cub. centims. were titrated at once, 100 cub. centims. warmed with invertase for 23 hours, and 100 cub. centims. boiled for two hours with 2 per cent. H_2SO_4 in a flask provided with an inverted condenser. This was then neutralized. After titration the weights of the CuO were found to be as under.

	100 cub. centims. original extract.	100 cub. centims. + invertase.	100 cub. centims. + acid.
Gross	1·3245	1·375	1·435
Tare	1·251	1·246	1·251
	·0735 or ·2205 in the whole	·129 or ·387 in the whole	·184 or ·552 in the whole

The difference between—

(2) and (1) is ·387 — ·220 = ·167 = CuO due to inversion of cane sugar.

(3) ,, (2) ,, ·552 — ·387 = ·165 = CuO ,, grape sugar formed from maltose.

As 1 grm. of cane sugar inverted reduces 2·321 grms. CuO, ·167 grm. CuO indicates ·072 grm. cane sugar present in the styles, or 2·1 per cent. of their dry weight of 3·403 grms. So, also, ·165 grm. CuO is equivalent to ·17 grm. maltose (·165 ÷ ·976 = ·17),* which gives 5 per cent. in the weight of the dried style tissue. The original reduction of the fluid is given by (1) and amounts to ·2205 grm. Now, ·17 grm. maltose would reduce ·228 grm. CuO, which agrees fairly well with this quantity ; glucose, therefore, was not present in the styles. Taking the sugars found in conjunction with the water evaporated, we have for the concentration of the sap ·21 per cent. cane sugar and ·49 per cent. maltose.

L. pardalinum.—105 styles were collected, found to weigh ·566 grm. when dried, and then treated as before. The final residue after solution in water was divided into two, and half warmed for 24 hours with an appropriate quantity of invertase. Both were then boiled with excess of FEHLING's fluid, and the CuO collected and weighed as in other cases. The weights were—

	Original solution	Solution + invertase.
Gross	1·279	1·292
Tare	1·249	1·249
	·030 or ·06 in the whole	·043 or ·086 in the whole

The increase in (2) = ·026 grm., which corresponds to ·0112 grm. cane sugar inverted, or 1·96 per cent. of the ·566 grm. dry weight of the styles. Also taking the reducing sugar in the residue as maltose, ·06 grm. CuO = ·0446 grm. maltose, or 7·9 per cent. of the same dry weight.

Besides these analyses of the styles of the two species of Lily, experiments were made with those of *Narcissus pseudo-Narcissus*, which proved to contain cane sugar, and a reducing sugar the nature of which was not satisfactorily determined. The cane sugar amounted to about 6 per cent. of the dry weight of the styles examined.

The quantity of starch present in styles of different ages was found to vary, the maximum observed being in those flowers which were just ready for pollination. As it diminished after this, and in old styles, whose attached ovaries were swelling into the stage of fruit, there was often but little to be found, it appeared possible that the style not only stores starch for the pollen tube, but may go further and present some of this at least to the advancing organ in the shape of the maltose found. Experiments were made to ascertain the presence or absence of diastase in the style, as well as in the pollen grain. The species taken for these experiments was *L. auratum*, the season of flowering of the others being over. Two stages were selected, one from flowers

* *Cf.*, p. 403.

which had been fertilized and showed the fruit in course of formation; the other from those whose stamens were just mature.

To secure the action of all the enzyme that might be present, the fresh styles were bruised to pulp in a mortar, and the pulp mixed, as it was, with the usual thin starch paste (1 per cent.). Half of each mixture was then boiled for several minutes, and the four quantities were allowed to digest in the incubator at 40° C.

Action was noticeable in 21 hours, the unboiled tubes having become limpid, while the controls were opalescent, as at first. After four days the difference in colour in samples treated with iodine was very marked, and the action was thereupon stopped. The digestions were then boiled with excess of FEHLING's solution, and the CuO determined as usual. The older styles gave a reduction which, when divided by the number of styles taken, amounted to ·0045 grm. CuO each; the younger, similarly computed, reduced ·014 grm. CuO each. Diastase consequently appears to be present normally in the styles, side by side with the starch, the quantity diminishing after fertilization.

Invertase was also tested for in a similar manner, but none could be detected.

In the course of development of the pollen tube, we have thus clearly two stores of reserve material for its nutrition. Part is deposited in the pollen grain itself, its nature and amount varying considerably in different plants. The grains also contain at some time or other the enzymes necessary for the transformation of these reserves into plastic material. At the same time, and particularly in styles which are of some length, the style contains a subsidiary store, part of which is transformed by enzymes also present in the style, and part by the excreted enzyme of the pollen tube. The action of the diastase is partly intracellular, as shown by the gradual transformation of the starch granules as they pass down the tube, and partly extracellular, hydrolysing the starch granules in the cells of the style. The relative times of action of the two portions of the diastase are indicated by the locality of the distribution of the starch in the latter, none being present in the portion just below the stigma. The extracellular action of the invertase is evident when we remember VAN TIEGHEM's observation that cane sugar is not in the pollen of Narcissus, while we have seen that it exists in the style.[*]

The secretion of enzymes in this case does not appear to be a starvation phenomenon, as noted in many other cases, especially in the hyphæ of Botrytis,[†] which show in many respects a similar mode of growth to that of the pollen tube. On the contrary, well-nourished grains show a greater formation than starved ones, indicating that the absorption of food material is a strong stimulant, much as has been determined in the case of the peptic secretion of the stomach. Pollen of Narcissus allowed to germinate in water did not yield so much invertase as the same quantity germinated in cane-sugar solution, the proportion being as 13 : 24.

[*] Compare pp. 403 and 406.
[†] MARSHALL WARD, loc. cit.

In other experiments on the same point the absorption of sugar led to a still larger increase. In *Zamia* no enzyme could be detected in the resting grains, but, on the absorption of cane sugar or glucose, even before visible extrusion of the pollen tube had taken place, a small amount of diastase was found to have been secreted. The temporary decrease of diastase noticed in the case of *Lilium pardalinum* as germination began may perhaps be explained by the assumption that the transformation of some of the reserve starch of the grain takes place before the protrusion of the tube, and that this involves a partial consumption of the enzyme. The secondary increase would then follow the absorption of food material from the culture medium as soon as the thin-walled intine allowed this to take place. At the same time it must be mentioned that the increase is not altogether dependent on such nutrition, for there was an increase of the quantity in grains germinating in water only (see p. 393).

Whether the enzymes exist in the pollen grains in the state of zymogen is a question of some interest. Only a little evidence was obtained on this point, drawn from a study of the *Zamia* pollen. The culture medium which best suits this pollen is, as already shown, the expressed juice of the apple or pear. Even the cane-sugar solution gives less satisfactory results than the juice, or the pulp of the fruits. As these juices both contain malic acid, we have in them just the condition needed to transform zymogen into enzyme. A small quantity of pollen was extracted with chloroform water for two days and filtered. Half of it was then made faintly acid with malic acid and warmed for twenty-four hours in the incubator. After careful neutralization with very dilute ammonia the two were mixed with starch paste, and half of each mixture was boiled to serve as a control. After forty-eight hours' action the four tubes were tested with iodine, when the one that contained the extract that had been warmed with acid showed very slight evidence of diastatic action; the one with the extract as prepared showed none. The controls were both unchanged. The quantities used in the experiment were only small, and the experiment can hardly be taken as certain evidence on the point, though the results were confirmatory of the hypothesis so far as they went. More experiments on the point are, however, necessary.

The course of events in the germination of the pollen grain appears to be the following. When it falls upon a suitable substratum, it absorbs water from the moist surface of the stigma, and swells, becoming generally more granular. This is followed by the absorption of whatever food material may be present. In presence of the water, intracellular digestion of the reserves at once begins and the ferment is rapidly increased after absorption of food. In some cases this increase only takes place after a temporary diminution, but ultimately a much larger quantity is present than at first. Very soon an excretion of the enzyme takes place, the reserves of the style thenceforward being attacked and affording the tube the plastic material for its further growth. In many pollens the absorption of the sugar is followed by the temporary increase of the starch in the tube, notably so in the case of *Zamia*, which contains none at starting.

At the same time the style takes part in the nutritive processes, by itself transforming part of its starchy reserves.

Though it would, perhaps, be at present premature to say that the process of pollen germination is altogether dependent on the presence of enzymes in the ripe grains, it is a very significant fact that as the grains lose their power of germination with increased age, this loss of power is attended by a very marked diminution in the quantity of diastase that can be extracted from them.

Summary.

The results of the above-described experiments may be briefly summarized as follows :—

1. Diastase and invertase are both present in pollen grains, and can be extracted from them by the same treatment as has been found effectual in the cases of seeds and foliage leaves. The relative quantities differ a good deal ; while some pollens contain both, others possess only one, which may be either of the two. Various solvents may be used for extraction, 5 per cent. solution of NaCl being the most generally useful.

Though the presence of a cytolyst is suggested by the growth of some pollen tubes, it has not yet been demonstrated.

2. At the onset of germination, usually the amount of both diastase and invertase is considerably increased. In one species examined, this increase was preceded by a primary diminution. When the pollen grain has lost the power of germinating the quantity of diastase has materially decreased.

3. The pollen tube is nourished during its growth by plastic material derived from two sources, the store of reserve matter deposited in the grain itself, and a further store deposited in the style.

4. The reserve store of the pollen grain consists of different materials in different species ; starch, dextrin, cane sugar, maltose, and glucose being the forms in which it is found.

5. The store in the style consists usually of the same carbohydrates, with the exception of dextrin.

6. The style itself contains enzymes to assist in preparing the reserve materials for absorption by the pollen tube, while the latter excretes the same ferments during its progress down the conducting tissue.

7. The absorption of food materials appears to be one cause of the increase of enzyme found to occur during the germination.

8. The absorption of food material is usually so active that the reserve store of the pollen grain is often largely increased by a temporary deposition, either in the grain or its tube, of some of the absorbed sugar in the form of starch.

9. There is a certain amount of evidence pointing to the existence of zymogens in some pollens, particularly such as germinate best in a faintly acid medium.

XI. *The Menstruation of* Semnopithecus entellus.

By WALTER HEAPE, *M.A., Balfour Student in the University of Cambridge.*

Communicated by Professor MICHAEL FOSTER, *Sec. R.S.*

[PLATES 35–41.]

CONTENTS.

INTRODUCTION.

DURING a visit to India in 1888–89 I passed through Calcutta, and made inquiries from the Superintendent of the Zoological Gardens there, Mr. SÁNYÁL, as to the breeding time of *Macacus rhesus.*

I was informed they bred freely in the gardens all the year round, and that full-grown specimens were readily obtainable in the bazaars.

In consequence of my representations to this effect, the Managers of the Balfour Memorial Fund handed over to me the sum of £100, for the purpose of investigating in this country the embryology of Monkeys.

Through the kindness of Mr. SÁNYÁL, forty female *Macacus rhesus* were purchased and shipped in Calcutta, and arrived in England in March, 1890; and it was with great disappointment I found they were too young for my purpose.

I was then informed that adult *M. rhesus* are so savage it was found impossible to send them so long a journey without providing a separate cage for each, and I therefore determined to go to Calcutta to carry on researches there.

The Committee of the Royal Society appointed to administer the Government Grant for the endowment of research gave me, in October, 1890, the sum of £100, for " an investigation of the phenomena of menstruation and ovulation, and of the early stages of development of the Monkey." I was elected Balfour Student in the November following, left England the same month, and arrived in Calcutta in the middle of December.

My object in visiting Calcutta was placed before the Committee of Management of the Zoological Gardens by Professor D. D. CUNNINGHAM, the Secretary, and these gentlemen most kindly placed at my disposal a small building within the gardens, which was converted into a temporary laboratory.

I should like to take this opportunity of expressing to these gentlemen my thanks for their courtesy, and to thank especially Professor CUNNINGHAM for his never-failing willingness to put at my disposal all the accurate and varied knowledge he possesses, and for very many acts of kindness which were of the greatest service to me. The varied religious beliefs of the natives, the fanaticism of some of them, and the special reverence accorded to Monkeys by many, rendered secrecy and some considerable caution necessary; it was in this direction, and in overcoming the difficulties which presented themselves of obtaining a sufficient number of satisfactory animals to work upon, that Professor CUNNINGHAM's advice and help was of especial, and indeed essential, importance.

Finding that none but very young Monkeys were obtainable in the bazaars, collectors were dispatched to the jungle to secure as many specimens as possible of adult female *Semnopithecus entellus* and *Macacus rhesus*.

The latter were not obtainable near Calcutta in this way, and in spite of daily promises from the dealers, it was not until the middle of January that *S. entellus* arrived in numbers from the jungles on the south bank of the Hugli. Subsequently, large numbers of *M. rhesus* were sent to me from the North-West Provinces, where the protection afforded by numerous Monkey temples and worshippers had favoured their increase to such an extent that some of the inhabitants were very willing to assist in capturing and sending away as many as possible,

Of the one hundred and eight specimens of *S. entellus* examined, a considerable number had already borne young, and were suckling them; six were found to have very lately borne young, and one to be undergoing the process of aborting an advanced embryo; the rest were not breeding, and about fifty of these, adult females, were killed, and the uteri preserved, in order to study the phenomena attending menstruation.

I was informed by the dealer—a Eurasian—who supplied me with these animals, that *S. entellus* breeds twice a year, in April and October, and that, when breeding, they retire into the thickest parts of the jungle, and cannot then be caught. This information was doubtless obtained from native collectors, and all evidence supplied by natives was found to be so untrustworthy that little reliance can be placed upon this report.

Bearing in mind the·fact that the animals examined were either not breeding, or else had recently borne young, it would seem fair to assume there are one or more limited breeding seasons for *S. entellus*, but my information warrants no further assumption.

I made attempts, during February and March, to induce these animals to copulate while in captivity. Two large cages were put at my disposal in one of the Monkey houses at the gardens, and an adult male with several females put in one cage, while in the other a pair of adult Monkeys were kept.

Many of the females seemed quite prepared for copulation and tried to induce the male to fertilize them, but without success.

I do not consider these observations at all conclusive towards establishing the fact that the animals in a wild state do not breed during these months; *S. entellus* is a very shy animal, and the circumstances of captivity may well account for their non-breeding in the cages; at the same time, if the breeding season had begun before I left, it is highly probable I should have seen some evidence of the fact in one or more of the numerous specimens I examined.

With *Macacus rhesus* I was even less successful in obtaining material for work.

Of several hundreds of these animals sent to me from the North-West Provinces during February and March, a very large proportion, probably not less than four-fifths, bore advanced embryos in utero, or had lately borne young, or had recently aborted the embryo.

In two specimens I found embryos of a stage probably similar to a six-weeks-old human embryo, and these were the only specimens of breeding females I saw which bore any but nearly full-grown embryos. The rest of the females were mostly young ones, but about twenty-five were adults, not pregnant, and the uteri of these were preserved for the investigation of menstrual phenomena.

The large majority of breeding females which either bore young of an advanced age *in utero*, or which had recently borne young, is strong evidence in favour of the assumption that *M. rhesus* has one or more definite breeding seasons; Mr. SÁNYÁL,

however, assures me he has had various specimens of this species in the gardens, which gave birth at different times of the year, and I can only assume it is probable the species breed at different times in different parts of the continent.

The term of embryonic life of *M. rhesus* was calculated by FRÉDÉRIC CUVIER (quoted by BRESCHET, No. 5A) to be seven months ; the female upon which he carried on his observations giving birth to one young one in the month of October.

Dr. J. E. T. AITCHISON, however, assures me that in Simla *M. rhesus* copulates about October and gives birth during August and September following. The time for copulation, he adds, doubtless extends over two months. This would indicate the term of embryonic life to be nine to ten months, and Dr. AITCHISON's observations are so circumstantial I have no hesitation in accepting them.

It follows that *M. rhesus* of the plains of the North-West Provinces, which give birth during February and March, begin to breed about May. The great difference in the climate of the hills and the plains may be sufficient to account for the different breeding times in the different districts, and, as I have already pointed out, the fact that such different breeding times do exist, is substantiated by the experience of Mr. SÁNYÁL.

My stay in Calcutta was cut short by an attack of rheumatism and fever, and in April I was advised to leave the country as soon as possible. On this account I was compelled to defer my researches on the embryology of Monkeys, and to confine my attention to the menstrual phenomena.

In this paper I propose to describe the histological changes taking place in the uterus of *Semnopithecus entellus* during menstruation, and before long I hope to supplement these researches by an account of the same process in *Macacus rhesus*.

METHODS.

The methods adopted for the preservation of the uteri were—
Fixing :—

1. In PERENYI's fluid, for 4 to 5 hours.
2. In a saturated solution of corrosive sublimate, for 3 to 6 hours, after which careful washing in water intervened before hardening.
3. In FLEMMING's solution, without acetic acid, for half an hour.
4. In FLEMMING's solution, with equal volume of ·5 per cent. osmic acid, from half to 2½ hours.
5. In osmic vapour, for 5 minutes to half an hour, followed by FLEMMING's solution, either with equal volume of ·5 per cent. osmic acid added, or without the acetic acid, from half to 20 hours.

Hardening with spirit followed, attention being paid to a gradual increase in the strength of the spirit, and the specimens were kept in 70 per cent, or 90 per cent, spirit, until required.

The tissue was embedded in paraffin, and cut, by the Cambridge Scientific Instrument Company's rocking microtome, into sections of a thickness varying from ·0025 millim. to ·00625 millim. in different series.

The combination of soft mucosa tissue and thick, hard, muscular tissue caused much trouble by a folding or wrinkling of the sections, as they were cut, and it was not until several methods had been tried to get rid of this fault that I devised a plan of running hot water on to the slide upon which the sections were to be mounted, through a pipette, and placing the sections on the water; the latter is used at a temperature sufficient to soften the wrinkled paraffin and cause it to straighten out, but not quite hot enough to melt it.

The slide is previously coated with a thin layer of MAYER'S albumen, and when the water is run off, by tilting the slide, the sections sink down on to the albumen. The slide is then put on to a water bath, until all trace of water is lost by evaporation, and the sections become fixed in the hardened albumen.

By this method, several rows of consecutive sections can be mounted on one slide, which is then treated with turpentine, spirit, and staining reagents, then back through spirit to xylol, and mounted in Canada balsam in the usual way.

I used various stains with good results, especially dahlia, eosin, methylin blue, hæmatoxylin, EHRLICH'S Biondi gentian violet, borax-carmine, and picro carmine, methylin blue and eosin, hæmatoxylin and eosin, saffranin, or fuchsin. After using picro-carmine, the slides were passed through spirits of various strengths in which a little picric acid had been dissolved, and this stain was perhaps the most successful of all for general work, but for finer histological examination the other stains, especially gentian violet and hæmatoxylin and eosin, were more advantageous.

The sections were cut through the body of the uterus, at right angles to its antero-posterior plane and vertical to the wall of the uterus. It will be observed that in some of the figures the mucosa is not so deep as it is in others, and I should remark that, where it is more shallow, the drawing has been taken from the lateral part of the section, where (*vide* fig. 12) this layer is considerably less thick than it is in the middle of the layer.

SUPERFICIAL MENSTRUAL PHENOMENA.

After histological examination, the uteri were grouped into the following four menstrual periods, which were subdivided into eight stages, and, as it will be convenient to describe the superficial phenomena in relation to these periods and stages, I will mention them now.

A. Period of rest.
  Stage I.—The resting-stage.
B. Period of growth.
  Stage II.—The growth of the stroma.
  Stage III.—The increase of vessels.

C. Period of degeneration.

    Stage IV.—The breaking down of vessels.

    Stage V.—The formation of lacunæ.

    Stage VI. --The rupture of lacunæ.

    Stage VII.—The formation of the menstrual clot.

D. Period of recuperation.

    Stage VIII.—The recuperation stage.

The external phenomena attending menstruation in *S. entellus* is marked by the discharge which flows from the vagina during about four days each month.

Unlike *M. rhesus*, there is no vivid colouring of the buttocks, stomach, thighs, or tail, and the only other external sign of menstruation is a slight swelling of the vulva, and, sometimes, of the nipples of the mammæ.

The dark colour of the labia and of the nipples prevents any external sign of flushing being seen, but just within the labia the skin is seen to be flushed during the menstrual period.

I have not examined the discharge from the vagina of *S. entellus* further than to determine the presence of a slimy white matter—probably mucus, of cells resembling pus cells, of red blood corpuscles, and of débris of cells both epithelial and belonging to the stroma layer of the uterus and squamous epithelial cells from the vagina.

The climate of Calcutta, and the fact that the discharge occupies some time in travelling down the vagina, made it difficult, if not impossible, to obtain material in a condition to repay study. I have, however, several menstruating *M. rhesus* now in my possession, and hope before long to publish the results of examination of the menstrual discharge in these animals.

As soon as the Monkeys were killed, the uterus, with part of the vagina, the tubes, and ovaries were cut out altogether. The uterus was then opened from the external os uteri along the left and anterior borders, and the ventral wall was turned back.

The vagina is a wide sac with very thick muscular walls, much folded on their inner side and lined with a thick layer of squamous epithelium. Projecting into the vagina at the further end of the sac is a small papilla, in the centre of which is a small hole leading into the cervix of the uterus. The lips surrounding this hole are soft, and, as the papilla is more closely attached to the ventral wall of the vagina than it is to the dorsal wall, the dorsal lip is longer and looser than the ventral lip.

The cervix is a narrow, straight canal, the walls are very thick and muscular, and its inner surface is longitudinally folded; it opens into the cavity of the body of the uterus by a gradually widening aperture.

The cavity of the body of the uterus is triangular in shape, all the sides of which are concave, the apex is at the cervix end, and the concave base is formed by the anterior wall of the fundus; it is lined by a mucous membrane, which I have called

the mucosa. The Fallopian tubes open by small pores on either side at the widest part of the triangle.

A superficial examination of the mucosa during Stages I. and II. shows the surface to be of a more or less opaque white colour, either smooth, slightly ridged, or divided into polygonal areas by pits connected by narrow depressions.

The opacity is more marked in some than it is in other specimens, and is regulated by the density of the stroma layer below the epithelium layer of the mucosa (fig. 12).

The smooth ridged or reticulate appearance is likewise due to the growth of the stroma. The reticulate appearance is brought about by the growth of the stroma between the glands; it rises like a number of small hillocks having valleys or depressions connecting the widely open mouths of the glands one with the other. In those uteri in which the mucosa is ridged, the epithelium has also grown and allowed the swelling stroma below it more room to expand; and where the mucosa is smooth the mouths of the glands are very small, and the interglandular tissue is evenly swollen all round them.

Throughout the process of menstruation, whenever an epithelium is present, the surface of the mucosa presents one or other of these three appearances; generally it is ridged or folded, but it may be smooth, or more rarely reticulated.

As shown above, these appearances are due to the growth of the stroma, restrained, as it were, by the epithelial covering. Where most restraint is exercised, hillocks or ridges are formed; where free growth is allowed the mucosa is smooth.

The soft mucosa is dry, and there is no trace of discharge from the glands within the cavity of the uterus during Stages I. and II. During Stages III. and IV. the mucosa is more or less flushed, the flush occurring uniformly over the surface when the mucosa is smooth, but more concentrated at the top of the ridges when the mucosa is folded.

At the end of Stage IV., at which stage, as will be seen later, the superficial capillaries of the mucosa break down and the blood contained therein is scattered among the meshes of the stroma, this flushing becomes exaggerated into congestion; and at Stage V. dark red spots are to be seen scattered about all over the surface of the mucosa: they are contained within the epithelium, and are caused by the formation of lacunæ.

It is to be noted that when only few specks of blood are seen they are confined to the dorsal wall of the uterus, or are more numerous there, and it is only when they are plentiful that they occupy both walls in equal proportions. This circumstance indicates that the increased supply of blood, which is a marked feature during menstruation, affects the dorsal before it affects the ventral walls, a supposition which is confirmed by histological examination.

The fact that during pregnancy the dorsal disc of the bilobed placenta is developed faster than the ventral disc is in harmony with this statement.

In the cavity of the uterus there is no discharge from the glands during Stage III.;

during Stages IV. and V., however, a colourless viscid material is sometimes seen therein; the labia, also, have a viscid discharge adhering to them, and I am inclined to think much of this is derived from the glands of the cervix.

The discharge in the uterus appears under the microscope as a stringy non-cellular substance; that taken from the labia contains, besides, many squamous epithelial cells derived from the vagina.

Stage VI. presents a further development; although no rupture of the surface can be discerned by a superficial examination, the cavity of the uterus contains free blood, which evidently proceeds from the mucosa, and, as will be seen, is expelled into the uterus by the breaking down of the epithelium covering the lacunæ, thus setting free the blood contained therein.

The lacunæ do not all rupture at the same time; free blood is found in the uterus, while specks of blood, i.e., lacunæ, are still to be seen in the walls.

The blood is thin and mixed with viscid material; it contains, also, epithelial cells. The os uteri is generally softened.

Stage VII. shows the formation of the menstrual clot; and now the surface of the mucosa presents a ragged and torn appearance, due, as will be seen, to the casting off of the superficial part of the mucosa. The clot itself is found to consist of great quantities of red-blood corpuscles, leucocytes, epithelial cells both from the epithelium covering the surface of the mucosa and from the epithelium of the glands, and great masses of stroma.

The size and consistency of the clot varies in different specimens. In some specimens it is small, stringy, and soft, in others it is large enough to distend the narrow cavity of the uterus, and to swell out the walls, in which case it is much harder, and approaches the consistency of putty when ready for use. The labia now are soft and flabby.

Stage VIII.—After the clot is naturally expelled, a process which takes place during this stage, the mucosa appears less ragged than during the formation of the clot owing to the re-formation of epithelium over the surface.

Free blood is still found in the uterine cavity for some time after the blood clot has been expelled, but only in small quantities, and a few epithelial and other cells are still found in the blood.

Gradually the ragged appearance of the mucosa gives places to a smooth surface, which is at first much flushed, but which becomes later a semi-transparent and then a more opaque white colour. At this latter stage the mucosa has returned to its resting state, only to be again disturbed after a brief interval by renewed growth, congestion, and rupture, as before.

Sections show the transparency observed at this stage to be due to the sparsely scattered nuclei of the stroma underlying the newly-formed thin and flattened epithelium (fig. 10).

The os uteri remains flaccid, and the labia are still swollen until towards the close of this stage.

HISTOLOGY.

General Description of the Body of the Uterus.

The whole of the cavity of the body and fundus of the uterus is lined with mucous membrane, called henceforth the mucosa. The mucosa consists of a single layer of cubical epithelium, below which is a tissue of a very primitive nature, which I have called the stroma, and about which a few words are here necessary.

The stroma is formed of a network of protoplasm in which nuclei are embedded; no definite cell boundaries are to be seen, the internuclear protoplasm is continuous, being drawn out into very fine processes; no intercellular substance is distinguishable and, with the exception of a few long radially arranged fibres which are present in the deeper part of the stroma during the resting stage only, there is no sign of connective tissue fibres or other skeletal structures.

At certain times the stroma grows rapidly, the nuclei increasing in number by division; the material which is cast off during menstruation is chiefly composed of stroma, and it is from stroma that the new blood vessels and some of the new epithelial cells, which are formed during the recuperation stage, are derived. It is, in fact, more like embryonic mesoderm than any tissue with which I am acquainted.

This stroma then is essentially primitive tissue capable of extensive and rapid growth, capable also of transformation into other and more specialized tissues, and may be entirely devoid of skeletal structures.

That portion of a mucous membrane which lies below the epithelium is usually called the corium, and, according to QUAIN (56), consists of connective tissue, either areolar or retiform.

JOHNSTONE (29) describes the corium of the human uterus as adenoid tissue, and considers it is a highly specialized form of connective tissue. CHROBAK (7) on the other hand finds no definite connective tissue framework in the mucosa of the human uterus, and I find practically none in the mucosa of *S. entellus.*

The nature of the so-called corium may be, and indeed is, different in different animals, in some it is denser than it is in others, while in others again a more or less definite connective tissue framework appears to exist.

In *S. entellus*, however, at any rate it does not consist of either areolar or retiform connective tissue, and the entire absence of fibres, except at one particular time, in my opinion, prevents it from being considered as definite connective tissue at all.

The mucosa then consists of a cubical epithelium below which is the stroma, the stroma contains blood vessels, a few radially disposed muscles in connection with the internal muscular coat, and long or short, generally straight and simple glands, the columnar epithelium of which merges into and is continuous with the epithelium lining the cavity of the uterus (see figs. 1 and 2, &c.). During Stage I. a few long fibres are present (fig. 1), but these are not present at any other stage.

The mucosa is thickest along the middle line of the dorsal and ventral aspects, and gradually becomes thinner laterally, leaving the cavity of the uterus as a triangular shallow slit the sides of which are concave.

The cavity itself is shallowest laterally, and widest at a point where the Fallopian tubes are inserted. The base of the triangle is formed by the curved wall of the fundus, the apex is at the entrance of the cervix into the body of the uterus.

Bounding the mucosa on its outer side is an inner layer of muscles mostly consisting of bundles disposed in an irregular circular direction, they run more or less obliquely round the mucosa, but at so small an angle that I have called them circular muscles ; besides these, and enclosed between the bundles of circular muscles, are bundles of longitudinal muscles ; but these are not so numerous as the circular bundles in this inner layer.

From the circular muscle bundles scattered fibres run radially inwards a short distance into the mucosa, they are not numerous and are irregularly placed, their existence, however, prevents the mucosa being sharply marked off from the muscle layer as MINOT (47) describes for the uterus of the human female.

Outside the inner layer is an external layer of muscles, chiefly composed of bundles of longitudinal fibres, but between them are smaller bundles disposed in a circular direction.

Outside the longitudinal layer of muscles is a thin layer, represented by a dark line in fig. 12, which is only superficially different from the external layer of muscles by reason of the fact that it does not stain so deeply.

It is composed of scattered fibres of longitudinal and circular muscles, with a few connective tissue cells, the whole embedded in a gelatinous material, and covered outside with a layer of flattened epithelium. I have called this layer the sheath.

All the muscles are non-striated muscles.

The cervix.—At the cervix end or posterior end of the body of the uterus, the stroma layer becomes thinner and gradually merges into a tissue in which the nuclei are more scattered and an intercellular substance is present. This tissue is much more dense than the stroma of the body of the uterus. The glands are short and the epithelium lining the cavity of the cervix is cubical.

I may here mention that neither the epithelium nor any portion of the underlying layer of the cervix is cast off during menstruation. During Stages II. and III., however, there is a slight increase in the density of the tissue at the anterior end of the cervix, but this is slight, and there is also a slight increase in the blood supply during these stages, but it is not at any time considerable.

The glands of the cervix secrete during menstruation, and I am inclined to think it is probable more secretion comes from them than from the glands of the body of the uterus.

The Fallopian tubes.—At the junction of the Fallopian tubes with the uterus the muscle layers of the latter are continued over the tubes, and a thin layer of stroma

underlies the layer of columnar epithelium, which lines the cavity of the tubes. Both these latter layers are continuous with the same layers of the mucosa.

I can detect no change in the structure of the Fallopian tubes during menstruation.

I will now give a description of the histological changes which take place from Stages I. to VIII. of menstruation.

A. Period of Rest.

Stage I.—The resting stage. Figs. 1, 13, 14.

During the resting stage the mucosa appears in section as of remarkably even consistency throughout.

The epithelial cells, either cubical or columnar, have large rounded nuclei in which a nuclear network is plainly visible with a high power. The epithelium is formed of a single row of such cells, their superficial edge is sharply delineated, but the inner edge is not so; there the protoplasm of the epithelial cells is continuous with the protoplasmic processes of the stroma which lies beneath, and indeed the similarity of the nuclei in these two layers and the continuity of their protoplasm point to the conclusion that the epithelium is merely a specialized layer of the stroma. The phenomena, which will be described later, of the re-formation of the epithelium after the mucosa menstrualis is cast off, although not absolutely conclusive evidence of this relationship, nevertheless renders it more than probable.

The uterine epithelium is directly continuous with the epithelium of the so-called uterine glands (fig. 1). These glands consist solely of columnar epithelium, which may be one or two rows deep, the cells are much elongated and their nuclei large, exhibiting a nuclear network. The superficial edge of the cells is usually beset in my preparations with ragged processes which may be cilia, but I have not been able to prove this to my satisfaction. The inner edge of the cells is very evenly disposed and attached to a non-nucleated basement membrane, which becomes thinner near the mouth of the gland and disappears altogether where the uterine epithelium joins the epithelium of the gland at its mouth.

There is a difference of opinion on this point with regard to the glands of the human uterus; ENGELMANN (11) says the glands of the fully developed human uterus have no basement membrane, MINOT (47) and LEOPOLD (38), however, state that a basement membrane is present.

No definite sheath invests the glands of *S. entellus;* close round the glands the nuclei of the stroma are more flattened than they are elsewhere during this stage. There may be one or more rows of such flattened nuclei, but the protoplasmic processes of the stroma do not combine to form a definite sheath, and they are continuous with the protoplasm of the surrounding stroma.

The protoplasm of the stroma does not appear to be continuous with that of the cells of the glands, as it obviously is with the uterine epithelial cells.

The glands secrete a clear viscid material, but at Stage I. this is rarely evident. During this stage the glands are generally short and their lumen narrow.

The nuclei of the stroma are regularly disposed for one-third of the depth of the mucosa; they are not closely packed, and the delicate protoplasmic processes of the stroma, in which granules are distinctly seen, form a network of open tissue, within which no intercellular substance was observed.

The nuclei are rounded or oval, and of very regular size; a nuclear network is always seen in them.

For one-third of the depth of the mucosa this arrangement is very uniform, but deeper down a few long fibrils run through the tissue, spreading out fanwise in the interglandular regions, and more closely concentrated at the base of the layer.

These fibrils stain darker than the neighbouring branching protoplasmic processes of the stroma, with which, however, they are in very close relation, a series of nuclei being disposed alongside each fibril.

The fibrils have not the structure of muscles, and are not continuous with the muscles lying below the mucosa; I judge them to be formed from united protoplasmic processes of the stroma, and to be similar to connective tissue fibrils; they were only seen in specimens of this stage of menstruation. Subsequently they entirely disappear, and it is remarkable that it is only that portion of the stroma situated superficially to these fibrils which undergoes in the next stage active growth, and becomes in the later stages cast off as the mucosa menstrualis.

The temporary presence of these fibrils is striking evidence in favour of the view, that we have here tissue of a very primitive character. It would appear that it is capable of developing into connective tissue, and, as I will show below, into blood vessels and epithelial cells; it is then not definite connective tissue, but primitive tissue, from which connective tissue and other structures may be derived.

There is no sign of the multiplication of epithelial cells or of the nuclei of the stroma at this stage.

The blood vessels in the mucosa are small. A few arteries are seen in the deeper parts of the layer, but only thin-walled capillaries in the superficial part.

The capillaries are fairly numerous, and contain plenty of blood corpuscles, while, now and then, but only rarely, a leucocyte is seen in a vessel.

It is noticeable that the blood vessels do not closely invest the glands in *S. entellus* as they do in the mucosa of many animals; on the contrary, they are specially noticeable in the interglandular tissue.

Of the muscle layers I need not say more than I have said already, since they do not concern the phenomena of menstruation as described below.

B. Period of Growth.

Stage II.—The growth of the stroma. Figs. 2, 15, 16.

Three changes are now seen in the mucosa. The first of these is a gradual increase in the density of the stroma in the superficial third of the mucosa; the second, an increase in the size of the blood vessels, and the third, an interglandular swelling of the mucosa into the lumen of the uterus in the form of ridges or hillocks.

1. *The growth of the stroma.*—The increase in density of the stroma comes about gradually. It is due to the increase in number of the nuclei of that layer by means of division, and to the fact that they are packed closely together. That is to say, hyperplasia occurs.

As I have before remarked, no definite division of this tissue into separate cells is possible, and the protoplasmic network is always continuous. When nuclei divide and separate, they carry with them a portion of the protoplasm which originally surrounded the parent nucleus, but this protoplasm is never thus entirely separated, it is merely extended, and no actual division of a cell is seen. In speaking of the finer histology of the stroma, however, I have sometimes referred to the individual nuclei with the protoplasm immediately surrounding them as cells.

The multiplication takes place by simple division of a nucleus into two, and probably also by fragmentation of a nucleus. Owing to want of space and consequent pressure, a very large proportion of the nuclei become elongated and spindle-shaped, while the protoplasmic processes of the cells are still branched, but in a longitudinal direction (fig. 15). These nuclei divide by simple amitotic division.

c_1 to c_5, fig. 16, are figures of five nuclei undergoing division into two; it is noticeable that the characteristic phenomena of mitosis are not discernible.

The cell marked c_3 is very commonly seen, but in no case have I been able to see polar stars or other details of karyokinesis. The same phenomena is shown in fig. 15, where also stages in the growth of the newly-formed nuclei are shown (d_2). The series d_1 to d_4, in fig. 16, shows stages in the fragmentation of the nuclei of the stroma; d_1 is a typical nucleus with its surrounding branched protoplasm, of Stage I.

The cells of this series are markedly different from those of the *c* series, owing to the division of their nuclei into three or more parts, and in the appearance of the protoplasm.

d_4 shows the fragments of a nucleus somewhat separated, but I have never seen the fragments draw away and carry with them the protoplasm which surrounded the mother nucleus, as is done when nuclei divide into two; at the same time, as will be seen later, such separation is probable.

The *d* series of cells is rarely seen, and then only on the edge of the denser layer of the stroma.

The cell indicated by f_1 is commonly seen in the midst of the dense layer. Its

three nuclei are not like those seen in d_3, nor are they quite like the two nuclei seen in the c series; at the same time, its protoplasm is much more like the c series than the d series, and I am disposed to think it should be classed with the former.

I at one time thought that these cells might be leucocytes, but have convinced myself this is not so. They have an entirely different appearance to leucocytes, their protoplasm stains much darker and their nuclei much lighter than do these parts in a leucocyte, and it is noticeable that no cells with four nuclei, which is the most usual condition of leucocytes at this and at other stages, are present in the stroma layer (compare *leu.*, fig. 16).

The same remarks apply to d_4 in the same figure; the appearance of the protoplasm itself and of the five nuclei therein is quite different from the appearance of those parts of a leucocyte.

The cells marked h_1 to h_5 are also very commonly seen in the dense part of the layer. They are very small cells and have very small nuclei; those represented in the figure were drawn with the same lens as the rest of the cells in fig. 16 (REICHERT's $\frac{1}{15}$ immersion and occ. 4). Their nuclei, also, are commonly seen to be undergoing division into two by simple amitotic division.

The origin of these cells I have been unable to determine, they may be either the product of the larger fusiform cells or they may be derived from those cells in which the fragmentation of the nucleus was observed; their size and the appearance of their nucleus strongly inclines me to the latter view, but there is so much difference of opinion as to the possible formation of cells in this manner that I must leave their origin an open question, merely insisting upon the fact that they are a new formation, and that they were not present during Stage I.

The occurrence of amitotic division or of fragmentation, and the entire absence of mitosis in the cells of the stroma, is remarkable. According to the researches of FLEMMING (14), and ZIEGLER (84), fragmentation does not lead to the reproduction of cells, but to degeneration, while, on the other hand, METCHNIKOFF's researches (45), throw grave doubts upon the destruction of polynuclear cells, and HICKSON (22, 23) shows that the nucleus of the ovum of *Millepora plicata* and of *Allopora* fragments, and that the cells of the blastoderm are formed of cells whose nuclei consist of the fragments of the nucleus of the ovum.

These researches are extended in a paper by the same author (24), and Dr. HICKSON informs me that in a forthcoming paper he will refer to some forty instances recorded by other writers, showing or implying the same thing.

Should this account be true, the origin of the h series of cells (fig. 16) from cells in which fragmentation of the nucleus is observed (the d series), is by no means improbable.

With regard to the occurrence of amitotic division, ZIEGLER (84) states that those nuclei which divide without mitosis are always distinguishable by their excessive size, and he connects the large size with increased functional activity.

My observations are not in accord with ZIEGLER'S; the stroma cells of the non-pregnant mucosa of *S. entellus* are at no time possessed of large nuclei, while during the period of activity just described, many of the nuclei exhibiting amitotic division are excessively small (series *h*, fig. 16), their size varies from ·005 to ·0075 millim.

It is not uncommonly remarked where no karyokinetic figures are observed that the failure to see them is due to faulty preservation or unsatisfactory staining; that is, of course, possibly the case with my own preparations, but I venture to think it is an improbable explanation in this instance. Possibly the relative size of the nucleus and the cell protoplasm may affect the method of division (figs. 16 and 17); in the examples before us there would seem to be no room for the formation of polar stars or nuclear spindle.

In the deeper two-thirds of the mucosa the stroma remains in the same condition as it was in Stage I., except that no fibrils are present; division of the nuclei is not seen, they are not crowded together, and hyperplasia does not occur.

Alongside the glands also the tissue is more open than in the interglandular regions (fig. 2).

I find no giant cells such as LEOPOLD (38) describes in the human menstruating uterus, and like WYDER (83) and MINOT (47), I do not find any decidual cells.

2. *The growth of vessels.*—The blood vessels in the deeper part of the mucosa are bigger in Stage II. than they were in Stage I., and the enlargement follows the first sign of growth in the stroma. The enlargement is not confined to vessels of the mucosa, those of the muscle layer are also enlarged, and further the vessels in the lower part of the mucosa begin to enlarge before the more superficial vessels are similarly affected, the increase in size gradually extending from below upwards.

In the densest part of the mucosa the vessels are still very small; pieces of capillaries from this region are shown in figs. 16, *a* and *b*, but a detailed description of the vessels will be given later, and I will not say more of them here.

3. *The swelling of the mucosa.*—The mucosa of the uterus from which fig. 2 was taken was swollen and thrown into folds; in that figure the swelling is seen to concern the interglandular substance and not to concern the gland itself.

The epithelium is very little, if at all, altered from what it was in Stage I.; the glands are firmly fixed deep down in a part of the stroma which undergoes no change at this time, and the growth of the stroma is greatest between the glands; in consequence of these facts the epithelium is held tightly down at the gland mouths and the swelling occurs where least resistance is offered by the epithelium, namely, midway between the glands.

The glands themselves remain short, but their lumen is wider than in Stage I.

Stage III.—The Increase of Vessels. Figs. 3–22.

A uterus at this stage of menstruation is readily distinguished in superficial examination by its flushed and swollen surface.

In section the cause of the flush is seen to be an increase in the size and number of the vessels directly below the epithelium, and to their congestion.

The epithelium itself is considerably thinner than before, the cells being somewhat flattened out though they are still cubical; this thinning is due to stretching, and there are now signs of cell division in this layer.

The nuclei of the stroma which were very densely packed in the preceding stage are now somewhat less dense, not on account of any decrease in their number, but in consequence of the increased room made for them by the stretching of the epithelium and its growth.

A reference to fig. 3 shows that advantage has been taken of this increased room, especially by those stroma cells lying directly beneath the epithelium; they have extended themselves and, in consequence, a layer of more scattered nuclei now intervenes between the epithelium and the denser layer below.

The thickness of the mucosa is thus increased in Stage III.

The blood vessels, which in Stage II. had begun to grow larger, are now larger still and more numerous. They have forced themselves through the dense layer of the stroma, reached the comparatively open tissue underlying the epithelium, and have there become enlarged to form flattened vessels which are gorged with blood.

The growth in size of the vessels is shown in fig. 22 to be due to the division and consequent increase of the cells forming the walls of the vessels. The vessels drawn in this figure lie below the denser layer of the stroma, but a similar increase in the cells of the walls of the more superficial vessels is also seen.

The increase in the number of the vessels, hyperplasia, may be a natural result following, and due to, hyperplasia of the stroma, according to ZIEGLER (85); and the congestion, to an increased flow of blood which is more marked in the next stage.

There is no change in the constitution of the deeper portion of the stroma, and no change in the glands during Stage III.

The number of the leucocytes in the vessels is increasing somewhat.

It is noticeable that many of the nuclei of the stroma in fig. 3 are smaller than they are in fig. 2. I have made a large number of measurements of these nuclei in different regions of the mucosa at the various stages of menstruation, with ZEISS' E. lens and occ. 3, and find there is a variation in size during Stages II. and III., a considerable number of the nuclei in the region of the denser portion of the layer being smaller than the rest. These small nuclei measure ·005 millim., while the usual size of nuclei during Stage I. is ·0075 millim.

The glands are much the same in length as they are in Stage II., but their lumen is still wider, and excretive action is apparent.

So far then, the changes which have taken place are changes due to the growth of the stroma or of the vessels contained therein.

C. *Period of Degeneration.*

Stage IV.—The Breaking down of Vessels.   Figs. 4, 17, 18, 19, 23, 24, 25, 37.

This stage, which is perhaps the most important and instructive of all, witnesses remarkable changes in the constitution of the mucosa.

Further swelling of the mucosa takes place, there is a growth of internuclear material of the stroma, and the nuclei which formed the dense layer of the previous stage become consequently more scattered. Simple hypertrophy follows, probably in consequence of the increased blood-pressure described in Stages II. and III. and which exists now to a greater extent (85), and degeneration supervenes in the superficial region of the mucosa.

The walls of the superficial vessels, which are dilated, rupture, and the red blood corpuscles, with which they are densely charged, escape and are scattered about in the network of the stroma. This extravasated blood is present below the whole of the uterine epithelium and causes a deep flush over the whole surface of the mucosa readily recognized with the naked eye.

The histological changes which take place in this stage are as follows :

The cells of the uterine epithelium increase in size and their nuclei become paler, the nuclear network less pronounced and frequently invisible, and the nodal points of the network combine to form a large, darkly staining nucleolus situated at the base of the nucleus (fig. 19).

The protoplasm of the epithelial cells is still continuous with the protoplasmic processes of the stroma.

The nuclei of the glandular epithelium exhibit less change, they become rounder than formerly and stain less deeply than they did during Stage I. ; a nuclear network, however, commonly exists, and there are several nucleoli, as formerly. The cells of the glands are the same as before and the basement membrane is present (fig. 18).

With regard to the stroma and its nuclei. The dense layer is still present in places and nuclei undergoing division, although much more rarely seen, still exist in this stage (fig. 17). As I have already mentioned, the mucosa is now still further swollen ; fig. 4 is taken from a region so much swollen that the dense layer has altogether disappeared, and here the change in the constitution of the protoplasmic material of the stroma can be most clearly seen.

The protoplasmic processes are not so definitely marked, and are more irregular than in Stage I. ; there is an increase in volume of the protoplasm, and a corresponding decrease in density and consistency (compare figs. 14 and 24).

The nuclei have also become larger than they were in Stages II. and III., they are more rounded, stain less deeply, and exhibit a nuclear network and many deeply staining nucleoli (compare figs. 15 and 24) ; indeed many of them are larger than those in Stage I.

These changes are undoubtedly of the nature of hypertrophy.

The greatest change, however, is noticeable in the cells forming the walls of the vessels, which also undergo hypertrophy. Fig. 23 is a drawing of an early stage of the process in a small vessel near the surface of the mucosa; the nuclei are much swollen and stain but lightly.

Fig. 24 shows a dilated capillary in an advanced stage, in which the nuclei and cells are still further enlarged, and in which the walls of the vessel have broken down, distributing the blood corpuscles amongst the network of the surrounding stroma. Both these drawings are taken from a uterus of Stage IV., from the region close to the uterine epithelium.

The hypertrophy of the vessel's wall is probably consequent upon increased blood-pressure (ZIEGLER, 85), while the rupture of the vessels is due to degeneration, and the decreased resisting power of their walls. A withdrawal of efficient support from the surrounding tissue of the stroma, brought about by the increased swelling of the mucosa, and the extension of the protoplasmic processes of the stroma, combined with their hypertrophy and degeneration, no doubt assist the rupture.

Further, there can be little doubt that similar changes are affecting the other parts of the mucosa, both epithelial and stroma tissue. It is very noticeable, however, that both hypertrophy and degeneration are most active in the superficial region, and that the deeper tissue is less and less affected in proportion to its remoteness from the surface.

The cells of the walls of the vessels in the deeper mucosa are hypertrophied (fig. 25), and strands of protoplasm project across the lumen of the vessels, but the degenerative changes are comparatively slight; these vessels do not break down, and no extravasated blood is found at this or any other stage of menstruation in the deeper regions of the mucosa.

Some authors, FEOKTISTOW, KUNDRAT and ENGELMANN, and WILLIAMS (13, 34, and 78), have ascribed the breaking down of the tissue of the mucosa to fatty degeneration; I have been quite unable to detect any signs of fatty degeneration in any cells of the mucosa at any period of menstruation. This conclusion has been arrived at only after careful examination of specimens preserved and stained in various ways (see Methods), and I have been compelled to reject the theory of fatty degeneration, and to adopt that of simple hypertrophy followed by degeneration, probably of an amyloid or hyaline type, as an explanation of the cause of the phenomena described above.

The size of the hypertrophied nuclei of the stroma may reach, during Stage IV., ·0125 millim., which is a very considerably larger size than the nuclei of the stroma of Stage I. attain to, namely ·0075 millim.

There is a decided increase in the number of leucocytes during this stage. They occur in the deeper vessels in greater numbers than formerly, and appear to be travelling, from some other region of the body, towards the surface of the mucosa by means of the vessels.

In the deeper region of the mucosa, where there is no extravasation of blood, no leucocytes are present outside the walls of the blood vessels, that is to say, migration of leucocytes does not occur there.

Superficially, where the blood vessels have broken down, leucocytes are found, together with red blood corpuscles, distributed within the network of the stroma; but, in my opinion, their presence there is not due so much to voluntary wandering as to their expulsion from the vessels by means of the sweeping action of the rush of blood.

My reason for this view is, that more leucocytes are seen adhering to the remnants of the walls of the broken down vessels than are seen in a free wandering state in the stroma (figs. 24 and 37). Indeed the occurrence of wandering leucocytes is rare, while leucocytes adhering to the remains of ruptured vessels are very frequently seen.

In METCHNIKOFF'S recent work on inflammation (45) he defines that process as a reaction of leucocytes against a dangerous element, and describes a congregation of leucocytes taking place at the required spot, and the absorption by them of the irritating element.

He adds that diapedesis only takes place when the element to be attacked by the leucocyte is outside the walls of the vessels, where that element is inside the vessel no diapedesis occurs.

At this stage of menstruation then, the phenomena exhibited, namely, swelling of the tissue, dilatation and congestion of the vessels and a congregation of leucocytes, indicates the existence of a noxious element in the blood at the surface of the mucosa, which is of an inflammatory nature.

The size of the glands is now somewhat increased, they become longer, apparently on account of the superficial swelling of the mucosa and not by downward growth, their lumen is wide, and active excretion is going on.

Stage V.—The Formation of Lacunæ. Figs. 5, 6.

The extravasated blood collects during this stage into lacunæ. The lacunæ are first formed (fig. 5) in the midst of the loose layer of the stroma, situated between the epithelium and the remnants of the denser portion of the former layer. The dense tissue still persists here and there, although of rare occurrence now.

Between the primitive lacunæ and the epithelium the stroma network is filled with extravasated blood corpuscles, and gradually, as more blood pours out of the broken vessels, the lacunæ increase in size, push aside the stroma tissue and arrive at the boundary wall of the mucosa, namely, the uterine epithelium (fig. 6).

Lacunæ so situated may be recognized, when the mucosa is examined superficially, by the presence of dark red spots scattered all over the surface, and they give to the mucosa its characteristic appearance during this stage.

It is noticeable that the lacunæ are formed in the mucosa of the dorsal wall of the

uterus earlier than in the ventral wall, which shows that the increased supply of blood affects the dorsal before it affects the ventral wall.

At this stage there are still vessels in the superficial part of the mucosa that have not entirely broken down; eventually they do so, but many are seen in figs. 5 and 6 with complete walls.

In the deeper mucosa all the vessels remain intact, and the veins in that region and in the muscle layer contain but little blood.

The only change which takes place in the stroma is the occurrence of certain nuclei, but few in number, which stain differently from the rest. They are generally rounded opaque nuclei which stain deeply, and are highly refractive; they are most frequently seen near the surface, and are never seen deep down in the mucosa.

A similar appearance is assumed by some of the free leucocytes.

I was unable to determine what this change might mean by examination of specimens of this stage only, but the occurrence of similar nuclei in the same region during the following stage, together with large numbers of stroma cells whose protoplasm is barely discernible, and whose nuclei are much shrivelled, leads me to believe they exhibit now the first changes due to degeneration. The nuclei of a few cells of the uterine epithelium are similarly affected, but with that exception the epithelium, whether surface or glandular epithelium, remains the same as in Stage IV.

The condition of the glands is the same as in Stage IV.

Stage VI.—The Rupture of Lacunæ.   Figs. 7, 20, 21, and 38.

The stage now to be considered exhibits the primary phases of denudation resulting from the preceding occurrences. The lacunæ now become very much enlarged, the degenerating epithelium which lies above them is greatly stretched and eventually ruptures, allowing the contents of the lacunæ to flow into the cavity of the uterus; but the lacunæ do not all rupture at once, and before a complete breakdown takes place blood escapes from them through small spaces in the epithelial outer wall (fig. 7).

In consequence of this, a superficial examination of the uterus discovers free blood in its cavity, while large spots of blood are still seen dotted about the mucosa.

In conjunction with the existence of these large lacunæ and the consequent· stretching of the epithelium, the glands at this stage are widely open, and in some instances their epithelium even is thinned.

The pushing inwards into the cavity of the uterus of the interglandular material, doubtless produces the force which pulls the walls of the glands widely apart, and the fact that the mouths of the glands are wider open than the lower part, confirms this suggestion.

It is very generally noticeable that the largest lacunæ are in the region of a gland, the lacunar space often running downwards alongside the wall of the gland for a considerable distance. In one of the lacunæ drawn in fig. 7, a whole gland is seen to

be included and surrounded by the blood space, and this is not an uncommon occurrence.

The vessels in the deeper mucosa remain intact without exception, and a few, but only very few, dilated capillaries with complete walls are seen close to the epithelium in places where no lacuna is present.

The lacunæ themselves have no regular lower wall, they are bounded on that side by the stroma; in some places the stroma processes appear to combine to form a wall which restricts further inroad of the blood into the tissue, but generally there is no such wall, and irregularly branching diverticula exist continuous with the main lacuna and containing extravasated blood.

Leucocytes are now more numerous both in the deeper situated vessels and in the extravasated blood, but the greatest number by far are found sticking to the broken down walls of ruptured vessels near the surface.

Colonies of leucocytes are not unfrequently seen within the deeper vessels, and nuclear reproduction appears to be vigorously progressing there (fig. 38); leucocytes with a single nucleus, some of them dividing, and with two, three, and four nuclei are indiscriminately seen.

I have never seen the division of the leucocyte cell, and FLEMMING (14) states the division of the cell itself does not usually take place. The occurrence of an increased number of these cells in the deeper vessels would indicate that the increased number in the superficial tissues is brought about by an increased supply from other parts of the body; on the other hand, the occurrence of small leucocytes together with larger ones suggests local increase (fig. 38).

With regard to the proportion of leucocytes to red blood corpuscles, there are 2 per cent. of leucocytes in vessels which are full of blood, while in ruptured vessels out of which blood has escaped there are 18·75 per cent. of leucocytes. This fact shows very clearly that, since they do not migrate in any numbers from the vessels, the business of the leucocytes lies within the vessels and not directly with the degenerating tissues of the mucosa.

The probability of the accuracy of this view is substantially increased by an examination of the percentages in Stage VIII., a record of which will be found in that section.

A further change now takes place in the superficial stroma and in the epithelial cells (figs. 20, 21). Some of the superficial stroma retains the appearance observed in Stage IV. (figs. 18, 24, &c.), but a large proportion now consists of nuclei much shrivelled and surrounded by little or no appreciable protoplasm.

These shrivelled nuclei stain very deeply, and it is with difficulty that any internal structure can be seen. I have, however, been able to determine the existence of the remnants of a nuclear network in many of them, in others it is not possible to do so.

Close beneath the epithelium forming the outer covering of a lacuna, isolated stroma cells are scattered, these are all in the same condition as those described

above, and are undoubtedly undergoing degeneration (fig. 21); below the lacunæ however the tissue contains normal as well as shrivelled tissue, while deeper in the mucosa the stroma has changed but little.

The highly refractile nuclei seen in the previous stage are now recognized to be nuclei undergoing the preliminary stages of degeneration, their identity with the shrivelled nuclei of this stage is demonstrable.

The nuclei of the uterine epithelium, especially where that layer covers a lacuna, are now also distorted in shape and of irregular size, while the protoplasm of the cells has become less dense and has lost entirely those processes which connect the epithelium with the network of the stroma.

The nuclei of some of the glandular cells near the surface are also shrivelled.

There is little doubt that in all cases the cells so affected are destined to be thrown off during Stage VII.

The glands remain the same as they were in the last stage; excretion continues to manifest itself in the contents of the glands.

Stage VII.—The Formation of the Menstrual Clot.   Figs. 8, 30.

During Stage VII. the full extent of the act of denudation is reached, the severe action disclosed being almost worthy of the term devastating.   All over the body and fundus of the uterus the superficial portion, about one-third, of the mucosa, including uterine and glandular epithelium, stroma and blood-vessels, is cast away, leaving behind a ragged wreck of tissue, torn glands, ruptured vessels, jagged edges of stroma, and masses of blood corpuscles, which it would seem hardly possible could be satisfactorily healed without the aid of surgical treatment.

When it is remembered that this extensive denudation of the mucosa is a periodic function in the adult animal so long as it remains unimpregnated, and when the various and complicated structures to which the mucosa gives rise when a fertilized ovum is present be recollected, it must be owned that we have here to deal with tissue of a most unusual character.

I am not aware of any organ or tissue in the animal kingdom which is subjected periodically to such a completely devastating action as menstruation causes in the mucosa, and none endowed with such powers of recuperation as that tissue manifests.

Fig. 8 is an accurate representation of a portion of a section through a uterus undergoing denudation.   The cavity of the uterus contains débris in the shape of pieces of glandular and uterine epithélium, masses of stroma and red blood corpuscles, and leucocytes, both fresh and degenerated; and there a clot is in the act of being formed from this débris.

The blood corpuscles are closely packed together, forming plastic cakes or lumps, and it is noteworthy there are no signs of threads of blood fibrin amongst the débris.

Many of the leucocytes in the uterine cavity are degenerated cells, their nuclei

being shrivelled and opaque, but living leucocytes are also seen, though not in a condition of active reproduction of their nuclei.

The percentage of leucocytes is now much more equal in the vessels and on the surface; in the vessels they exist at the rate of 3 per cent., and on the surface at 2·5 per cent., in comparison with the red blood corpuscles. This equalization is probably due to the fact that the ruptured vessels, to which, in the previous stages, a great number of leucocytes were found adhering, have been themselves cast off, and their contents mingled with the extravasated blood. A continued supply is, however, maintained in the vessels.

The stroma may be thrown off as scattered cells in masses, and the glands may be entirely ejected, though more usually only the superficial part of them is broken away.

The uterine epithelium is practically entirely destroyed over the whole surface of the mucosa; a few small pieces are seen in fig. 8, still retained *in situ*, but from their position and surroundings it is more than probable they will be swept away before the process is completed.

The portion of the mucosa thus cast off I propose to call the mucosa menstrualis, in accordance with WYDER's (82) suggestion, whose reasons for rejecting the term decidua menstrualis—namely, the absence of decidual cells in the menstrual mucosa—appear to me sound.

In the deeper part of the mucosa the stroma suffers no change, the blood-vessels there are still possessed of complete walls, and are larger and more numerous than before, and there is no sign of extravasated blood in this region.

Many of the stroma nuclei on the surface are shrivelled, and will doubtless be rejected eventually, the remainder appear like those drawn from a specimen of Stage VI., marked st_1 in fig. 20.

Near the surface, vessels with complete walls are very rarely met with in sections; most of the blood in that region, and there is no inconsiderable amount of blood still remaining within the mucosa, is contained in lacuna spaces, some of which are in direct communication with the cavity of the uterus, while others are completely enclosed by, and retained within, the stroma (fig. 8).

Those of the glands which remain deeply embedded are in a condition of considerable activity, if we may judge from the material contained therein, but they, also, usually contain blood and cast-off cells, which have been washed into them through the mouth. The necks of these remaining glands project into the uterine cavity, often unsupported by any other tissue.

Here the period of degeneration comes to a close; beginning with the breaking down of blood-vessels, it passes through the stages of lacuna formation, and culminates in the casting away of the menstrual mucosa.

D. Period of Recuperation.

Stage VIII.—The Recuperation.   Figs. 9 to 12, 26 to 29, 31 to 36, 39 and 40.

The history of the recuperation of the mucosa comprises an account of five important processes, namely :—

1. The re-formation of the epithelium.
2. The reduction of the blood supply.
3. The formation of new and recuperation of old blood-vessels.
4. The changes which take place in the stroma.
5. The behaviour of the leucocytes.

1. *The Re-formation of the Epithelium.*—This process begins before the menstrual clot is expelled from the uterus, and prior to the cessation of the flow of blood into the uterine cavity.

In the specimen from which fig. 9 is taken, a clot was present in the uterus, and fresh blood was flowing from the mucosa, but in various places on the uterine wall new epithelium was forming (*ep.ut.*).

Fig. 33 is an enlarged representation of some of that epithelium which is formed of flattened cells; it covers a stroma of scattered nuclei embedded in protoplasm, whose long, irregular protoplasmic processes are disposed more or less parallel to the plane of the epithelium, and the meshes formed by these processes are filled with red blood corpuscles and here and there a leucocyte.

These protoplasmic processes are directly continuous with the protoplasm of the newly-formed epithelial cells; the nuclei of the stroma and of the epithelium are remarkably alike, their structure is similar, they stain similarly, and, as far as I can discern, they are at this stage identical.

I have found it impossible to trace many pieces of such flattened epithelium to a point of origin from uterine and glandular epithelium already existing. As I will show directly, the torn epithelium at the mouths of the glands does give rise to some of the new uterine epithelium, but there is a large proportion of epithelium now formed, which I cannot convince myself is derived from any pre-existing epithelial structures.

In the drawing before us (fig. 33) there is a cell marked *y* ; this cell is undoubtedly included in the same layer with the cells forming the epithelium, and at the same time it is quite impossible to separate it from the stroma cell *x*, which lies directly below it in the figure.   The cell *x* is, without doubt, a stroma cell in close contact with three other stroma cells lying alongside of it, and its intimate relation with *y* makes it exceedingly probable the latter has been drawn into its present position from below.

Such instances as this are very numerous where isolated flattened epithelium is in

course of formation; sometimes a cell like x will be found somewhat more completely included in the epithelial layer, sometimes less completely removed from the rest of the stroma.

The transference of elements of the stroma to the epithelial layer is not, however, confined to such places as fig. 33 represents. Fig. 31 is a drawing of the growing point of a piece of epithelium, which is directly connected with the epithelium of a gland at its mouth. That end of the epithelial layer, marked *ep.ut.*, consists of columnar cells, and is directed towards the mouth of the gland, while, at the opposite end, the cells are flattened and the nuclei arranged longitudinally. Below the flattened epithelial cells and beyond them, is stroma tissue, which is so intimately connected with the former that it is quite impossible to determine to which layer it belongs now, or will belong in the future. Instances of this description are always very numerous, and increase the probability that stroma cells take part in the re-formation of the epithelium of the mucosa.

Other specimens, of which fig. 32 is an example, conclusively prove that some of the epithelium is formed by the division of cells already occupying that position. In this figure the nucleus of the terminal cell of a growing point of epithelium is seen in the actual process of simple division, and the relation of the stroma below, while showing connection with the epithelium, gives no indication of participation in the reproduction of that layer in the region from which this specimen was derived.

It is to be noted also in this specimen, that the nuclei of the epithelium, which are not yet columnar in arrangement, are closely packed, and have the appearance of having been produced *in situ* by division.

The further growth of the epithelium is shown in figs. 10, 11, 34, 35, and 36; during which the columnar arrangement is gradually resumed. At first (fig. 34) the nuclei are very irregular in shape, size, and arrangement; gradually, however (fig. 35), their arrangement becomes more uniform, and in fig. 36, which is taken from the same uterus from which fig. 10 is taken, they are but little different from the nuclei of the epithelium of Stage I. (fig. 14).

The cells marked d in figs. 34 and 35 are columnar cells, whose nuclei are undergoing simple division.

The so-called glands of the uterus are specialized portions of that epithelium which lines the cavity of the uterus, and as the deeper portions of the glands are not as a rule cast away with the menstrual mucosa, but remain firmly fixed in the deeper parts of the mucosa, their cells are at hand when the time comes for the re-formation of the uterine epithelium.

There is no doubt that a certain part of the new epithelium has its origin from the torn edges of the glands, but I have been able to assure myself that all the new epithelium has not such origin, and I have shown there are frequent instances of the occurrence of flattened epithelial cells on the surface of the torn mucosa, which are not connected with glandular epithelium, and which are derived from cells of the stroma;

further, I have shown that, at the growing point of epithelium already differentiated, stroma cells are probably drawn into the service, and assume the properties of epithelial cells.

The similarity of the nuclei in these two layers at the close of menstruation and during the recuperation stage, is perhaps further evidence in favour of my view.

I would here draw attention to a circumstance which may be considered to confirm the opinion that the stroma does give rise to epithelial cells.

There are, during Stage VIII., small masses of tissue not quite torn away from the rest of the mucosa, but hanging to it only by the protoplasmic processes of a few cells, and which consist of blood corpuscles and stroma cells. Some of these pieces are enclosed by the new epithelium, but some are not so enclosed. In the latter case the epithelium bores its way through the slender attachment, and so cuts off the dependent piece.

Two pieces of this description are shown in fig. 9 (marked x), and it is noticeable the outer layer of cells in one of these pieces has the appearance of flattened epithelium.

The outer layer of certain pieces is more marked than it is in others, but in all the same tendency is observed, and, in some, the likeness of the flattened outer layer to newly-formed flat epithelium is very remarkable.

Finally, the history of the development of the uterus shows that it is formed from the coalesced Müllerian ducts, and that the epithelium of the embryonic duct, which forms these parts, is at one time of a stratified character, becoming modified later into cylindrical epithelium (6 and 76).

This epithelium is derived from embryonic mesoderm cells in the embryo, from the same layer of cells, in fact, which gives rise to the rest of the mucosa.

It can, then, hardly be a matter of surprise to find, in dealing with a tissue which has so many primitive characteristics, that the new epithelium formed after the menstrual mucosa is cast off, is first of all formed of flat cells, and that its re-formation may be due largely to the specialization of cells from the same layer which, in the embryo, performed the same feat.

I should here draw attention to the absence, on the healing surface of the mucosa, of any pus. The multi-nucleated leucocytes, which give rise to pus, are either washed away with the menstrual flow, or included within the newly-formed vessels of the circulatory system.

2. *The Reduction of the Blood Supply.*—On this matter I have little to say. There is, undoubtedly, an escape of blood, while the menstrual clot is still in the uterus, and it appears that this flow is checked, if it does not altogether stop, when the clot is evacuated.

HENLE (21) shows that an involuntary muscle during contraction becomes anæmic, and that the result of the contraction of the muscles of the uterus, which are involuntary muscles, is a diminution of the blood flow. It would thus seem possible that the

contractions of the uterus performed in order to expel the clot would have the effect of checking the flow of blood to the organ.

Again, as we have seen, epithelium grows over the torn mucosa before the clot is expelled. I cannot say it grows over the whole surface, but it undoubtedly encloses a considerable part of the stroma while the clot is in utero, and, in so doing, prevents hæmorrhage.

These two circumstances combined, probably suffice to check the flow of blood, which the completion of the epithelium and the formation of new blood vessels entirely stops.

3. *The Formation of New and the Recuperation of Old Blood Vessels.*—The formation of new capillaries is one of the earliest signs of recuperation. In fig. 9, although there is still much extravasated blood in the mucosa, many new blood vessels have been formed in the superficial region; while in fig. 10 all the extravasated blood has been enclosed within vessels, with the exception of a few isolated red blood corpuscles scattered here and there in the tissue. Newly-formed capillaries are shown in figs. 26 and 28.

As I have already shown in Stage VII., during which hypertrophy of the stroma prevails, extravasation of blood corpuscles takes place. The corpuscles lie within the meshes of the stroma, in spaces which are to some extent filled up by the distended protoplasm during this stage. When the hypertrophied tissue recuperates the distended protoplasm is largely withdrawn, and the resulting processes are fewer, firmer, more dense, and stronger; at the same time this withdrawal of the hypertrophied protoplasm leaves angular spaces in which the corpuscles lie.

Such a condition is attained during the early period of Stage VIII. Subsequently, in those spaces in which the blood corpuscles lie, the angularity gradually disappears, and a regular rounded smooth-walled space is formed, continuous with certain neighbouring spaces similarly constituted.

The protoplasm of the cells bounding these spaces flattens out, the nuclei of the cells becoming also flattened and elongated, and numerous fine capillary vessels are thus formed, continuous in the deeper parts of the mucosa with larger pre-existing capillaries, and so with the circulatory system.

These fine capillaries exist only temporarily; when the blood corpuscles are again drawn into the circulation, and when the mucosa has shrunk again to its resting condition, the fine capillaries are no longer seen; but during the time in which the reclaiming process goes on they exist in very large numbers.

It appears that the only known mode of creation of new vessels in pathological formations is by the development of offshoots from the walls of existing vessels (85); QUAIN (56) describes the usual method of the formation of vessels in the embryo by canalization of connecting tissue cells, and BALFOUR (2) describes also larger vessels formed from solid cords of cells, the central cells forming the corpuscles, and the peripheral cells the walls of the vessels.

None of these methods is adopted in the formation of the new capillary vessels of the mucosa. They are undoubedly formed by enclosing intercellular spaces with the protoplasmic processes of the stroma cells; they are developed in countless numbers; wherever a solitary corpuscle is present in the midst of the tissue, there a vessel is formed, they cannot, therefore, be derived from the cells of the walls of vessels already existing.

With regard to canalization there seems no reason at all to suppose that the spaces in the network of the stroma are intracellular. They are undoubtedly intercellular spaces. BALFOUR states that GÖTTE (18) finds that the larger vessels in the Frog are formed as longitudinal spaces, the walls of which are derived from the indifferent cells bounding these spaces, which become flattened and united into a continuous layer. This is the only observation which I have been able to find which is at all in accord with the description given above.

There is no appearance of disintegration in the blood corpuscles which are retained in the tissue of the mucosa; their even contour and general appearance is the same as before the rupture of the vessels.

When the final stage of recuperation is reached (fig. 11) the vessels all over the mucosa are reduced in size (compare figs. 9, 10, 11), the minute branches described above (fig. 26) are but rarely seen, most of them have entirely disappeared, and the extravasated blood has been drawn again into the circulation.

Fig. 28 shows the appearance of a capillary situated near the epithelium, about the close of the recuperation stage.

As to the recuperation of the old vessels, a stage is shown in figs. 27A and 27B. The hypertrophied nuclei become reduced in size, and the swollen protoplasm of the cells is contracted and becomes more dense (compare fig. 25). The inner boundary of the wall of the vessel is now once more sharply delineated, and the vessel itself reduced to its normal size. The vessels in both these figures (figs. 25 and 27) were situated in the deeper part of the mucosa.

4. *The Changes in the Stroma.*—These changes are practically the same as those changes which reduce the cells forming the walls of the hypertrophied vessels to a normal size and consistency. Just as the swollen nuclei and cell processes of the vessels in fig. 25 become reduced to the proportions of those in fig. 27; so the enlarged nuclei and swollen protoplasm of the stroma, seen in fig. 24, give place to the compact, darker staining nuclei and fine thread-like protoplasmic processes seen in fig. 28.

This change is not simultaneous, nor, apparently, very rapid; cells which are still hypertrophied are seen in the midst of other cells which have assumed their normal size, and it is easy to trace the gradual return of the tissue to a resting condition again.

The multiplication of the nuclei of the stroma goes on to a limited extent, especially in the early stages of recuperation and near the surface of the mucosa (such cells are

shown in figs. 31, 33, 34, and 36, and marked *d*), but the amount of tissue so formed is not great.   The method employed is either constriction into two by amitotic division or fragmentation (fig. 29).

Here again my observations are at variance with ZIEGLER'S (84) conclusions, inasmuch as although the cells so formed constitute tissue which is probably destined to be cast off as the next menstrual mucosa, still that end will be preceded by a stage of very active reproduction ; hence, although the product of these cells may be destined to die, yet amitotic division in their case now, does not indicate the end of the series of division.

The effect of the reduction in size of the hypertrophied mucosa and the effect of the withdrawal of the extravasated blood into the circulatory system, naturally is to reduce the bulk of the tissue enclosed by the new epithelium.   In consequence of this reduction of bulk the tissue is at first very open, the stroma is then drawn together and the epithelium follows.

A stage is shown in fig. 10, in which the stroma has become more compact, leaving behind the thin layer of newly-formed epithelium, but retaining connection therewith by means of excessively long and very delicate protoplasmic processes.

The epithelium then follows, its flattened cells becoming converted into columnar cells, and thus increasing the thickness while decreasing the length of the layer.

The cells of the glands in the region of the mouth of the gland are similarly affected, as a comparison of figs. 10 and 11 will show.

A still more efficient means of reducing the length of the epithelium is a process of folding, which takes place in that layer on the surface of the mucosa and in the walls of the glands themselves.

This folding of the epithelium is shown in fig. 11, and it is by this means that new glands are formed, which take the place of those lost in the mucosa menstrualis.

It is worthy of note that these new glands are formed from newly-formed surface epithelium, as well as from epithelium which already functions as glandular epithelium.

5. *The Behaviour of the Leucocytes.*—The leucocytes seen in the earlier periods of Stage VIII. are very numerous, and it is a noteworthy fact they are, almost without exception, found congregated in the newly-formed capillaries, or included amongst the masses of red blood corpuscles which will eventually be enclosed within the circulatory system.

Isolated wandering leucocytes apart from red blood corpuscles, at this period are very rare indeed, and, after the new vessels are completed and all the extravasated blood reclaimed, I have never met with a wandering leucocyte.

The actual proportion of leucocytes to red blood corpuscles within the superficial vessels is much greater now than it ever has been before.   Fig. 40 is a drawing of a newly-formed vessel, and the leucocytes therein reach the proportion of 47·115 per cent.

This is not an isolated instance; I have frequently seen such vessels containing more than 50 per cent. of leucocytes.

In this stage, as in the earlier stages, when the leucocytes first became numerous, active division of the nucleus is going on.

The horse-shoe nucleus, the large single round nucleus, or the same dividing, the small nucleus either single or in the act of division, and leucocytes with two, three, or four nuclei, are all commonly seen within the vessels (fig. 39A).

In one specimen I saw a leucocyte whose large single nucleus was in the act of division into four by fragmentation (fig. 39B); this, however, is an exceptional specimen, the commonest are those represented in fig. 39A.

The absence of the phenomena of diapedesis, the absence of wandering leucocytes in either the deep or superficial layers of the mucosa, and the fact that leucocytes take no part in the re-formation of the tissue are points of no little interest, and require further notice.

COHNHEIM asserted that colourless corpuscles of the blood are a source of the new tissue which an inflammatory process may produce, and he is supported by various writers, mention of whom is made in a paper by SHERRINGTON and BALLANCE (65).

Amongst these perhaps the chief supporter of COHNHEIM'S view is ZIEGLER (86, 87). SHERRINGTON and BALLANCE also give a list of writers who deny to the migrating leucocytes any power of further development, and seek to show by their own experiments that COHNHEIM and ZIEGLER are wrong.

Since then METCHNIKOFF (45) has attempted to prove that mononuclear leucocytes are capable of transformation into epithelial cells, and quotes researches to prove that the nuclei present in polynuclear leucocytes may fuse together and form a mononuclear leucocyte.

My researches, hitherto carried on upon preserved tissue only, and studied by means of sections, cannot be utilised with the hope of making important additions to the knowledge which has been gained by the admirable investigations of the writers quoted above; nevertheless there are certain facts shown in my preparations which are not without interest.

The peculiarities of the nucleus or nuclei of leucocytes and the readiness with which they stain, make them very prominent objects in section, and serve to distinguish them readily from the cells of the surrounding tissue; so that I have some confidence in stating they do not appear, in my specimens of recuperating mucosa, to take any part whatever in the re-formation of that tissue.

As I have already pointed out, the greatest proportion of leucocytes are always found in the vessels, not in the tissue. Further, not only is there no evidence of diapedesis of leucocytes, but they show no signs of taking advantage even of ruptured vessels in order to migrate into the surrounding tissue; their presence outside the ruptured vessels appears to be due either to the force of the rush of blood which sweeps them from their hold upon the walls of the vessels, or to the fact that they

are scattered by the breaking up of the tissue itself. At all events their dissipation either causes their degeneration *in situ*, or the loss by the menstrual flow of all those which are not included again in the circulatory system.

Under these circumstances the presence of leucocytes in such large numbers would appear to be unnecessary.

According to METCHNIKOFF, the congregation of the leucocytes in the vessels near the surface, indicates the presence there of noxious material. The casting off of the menstrual mucosa together with this irritating substance, and the rapid, clean healing of the wounded surface would have the effect of clearing away that which the leucocytes were summoned to absorb ; their protective presence in this instance then is not required, and they may be said to have been induced to appear on the scene to undertake duties which are otherwise performed.

In a case of suppressed menstruation, possibly the leucocytes would play a very different part, and it would be a matter of great interest to determine what that part might be ; but where menstruation is normal and where there is a sufficiently complete denudation, it does not seem improbable that the necessity for the presence of the leucocytes is by this means greatly reduced, if not altogether removed.

If this suggestion is correct, the absence of wandering leucocytes in the tissues and the freedom from pus on the wounded surface is rendered more intelligible ; while the already noticed remarkable peculiarities which are observed during the process of menstruation are accentuated, and we have increased reason for the belief that we have here a recurrent process which is unique in the animal economy.

OVULATION.

The relation of ovulation to menstruation has long been, and still is, a matter of controversy, and I therefore paid some attention to the matter when removing the generative organs from the specimens of *S. entellus* obtained in Calcutta.

The results of these observations are as follow :—

In Stage I., of which stage six specimens were examined, no discharged follicles were seen in either ovary of any of them ; in three specimens there were prominent Graafian vesicles, and in the remaining three there were none visible.

In Stage II. there were also six specimens ; in none of them was there any sign of recent discharged follicles ; but in one specimen two old cicatrices were seen in one ovary. In four specimens prominent vesicles were seen in one ovary or the other, and in two there were none visible.

In Stage III. there were four specimens, of which one showed a red corpus luteum in the right ovary. The fimbriated extremity of the Fallopian tube was remarkably pronounced, and spread out as if it had been recently active ; but there was no sign of cilia on its epithelium, and no trace of an ovum either in the uterus or in the

Fallopian tube itself. The other three specimens had no discharged follicles; in one of them two prominent Graafian vesicles were present, but in the other two, none.

In Stage IV., of which there were six specimens, one showed a large pendent corpus luteum on the right ovary, and one showed evidences of cicatrices in the right ovary. Five specimens showed no discharged follicles, four of them no prominent vesicles, and two a few vesicles which were somewhat prominent.

In Stage V. there were five specimens, in none of which was a discharged follicle visible; in one there were, however, several small cicatrices in the right ovary. In three specimens there were more or less prominent Graafian vesicles, but in the other two none which were raised.

In Stage VI. there were five specimens also, one of which had an old corpus luteum in the left ovary, and one a cicatrice in the left ovary. None, however, had any recently discharged follicles; two were possessed of prominent vesicles, and the other three had none prominent.

In Stage VII. there were four specimens, in none of which was there a sign of a discharged follicle. Two of them had prominent vesicles and two none prominent.

In Stage VIII. there were twelve specimens; in none of them were there either corpora lutea or recently discharged follicles or cicatrices to be seen; in four of them there were prominent vesicles in one or other ovary, in four, semi-transparent spots could be discerned, and in the remaining four, none.

Besides these, in a specimen in which an embryo in utero was being aborted, there was a large reddish-yellow corpus luteum in the left ovary, and in two more specimens, which had recently borne young, there was a large corpus luteum in the left ovary of each of them; while in three specimens, which had borne young some time ago, and were then suckling them, no corpora lutea were seen, and no prominent Graafian vesicles.

Thus, as far as discharged follicles are concerned, it is seen that out of forty-two menstruating specimens, none of which had recently borne young, only two had a recent corpus luteum in their ovary. Such a result appears to me amply sufficient to warrant the statements :—

First, that ovulation does not necessarily occur during each menstrual period ; and

Secondly, that menstruation is not brought about by ovulation.

It will be observed, however, that the only two recently discharged follicles which were seen, occurred in specimens of Stages III. and IV. of menstruation; and it will be remembered that the first great increase of the blood supply to the uterine mucosa takes place at these stages. It is no doubt possible that this increased blood supply may affect the ovary, and may have a tendency, by means of pressure to induce ovulation where an ovum in a sufficiently advanced stage of development is present. In my opinion, this is a very probable view; it does not, however, permit of the inference that an ovum is actually dehisced at each menstrual period, but rather that

if an ovum in a sufficiently ripe state be present, the congestion which occurs during the menstrual period may cause it to be shed.

It is quite possible such a circumstance may occur in animals which menstruate and whose generative organs are gorged with blood at these times, though I do not consider there is any definite proof of the fact: at any rate, it is generally believed that in the lower Mammals "heat" is coincident with ovulation, and in these animals it would seem highly probable that the congestion which occurs during "heat" may bring about the rupture of ripe follicles in the ovaries.

With regard to the prominent Graafian vesicles, it may be urged that these vesicles are on the point of discharging the ova contained therein, and that they would have done so before menstruation was over.

During Stages II. to VIII., that is during menstruation, eighteen specimens were seen in which there were prominent vesicles in one ovary or the other, while twenty-two specimens, during the same stages, had no prominent vesicles at all. So that it would seem quite certain that twenty-two of these individuals, out of a total of forty, would not have undergone ovulation during the menstrual period then in progress, under any circumstances; and this evidence is again sufficient to show that ovulation and menstruation are not necessarily simultaneous processes.

But I do not believe that the prominence of a vesicle is any proof whatever of its complete maturity.

It is not at all unusual to find very prominent Graafian vesicles alongside of newly discharged follicles in Rabbits' ovaries 36 hours after copulation, at which time fertilized ova are found already part way down the Fallopian tubes, and I question very much the value of results which have been deduced from assumed knowledge of the degree of ripeness of a Graafian vesicle.

The evidence here offered then, points to the conclusion that the ripening of an ovum in the ovary is independent of the process of menstruation, and that ovulation is neither the cause nor the necessary result of menstruation. It is possible, however, that the increased blood supply to the generative organs during menstruation may induce ovulation when a sufficiently ripe ovum is present.

To what extent coitus may exert influence in the same direction I am not in a position to say from observations on *S. entellus*. PLAYFAIR (54) seems to think the stimulus of sexual excitement may cause the rupture of a ripe follicle; and certain observations I have made on Rabbits, lead me to think this view is possibly correct.

ACCOUNT OF RECENT LITERATURE AND CONCLUSIONS.

1. *Menstruation in Monkeys.*

On the subject of the menstruation of Monkeys, little has been hitherto written. RENGGER (62) observed a discharge from the vagina of a species of *Cebus* which

recurred at intervals of three, six, or ten weeks, the periods being irregular and the amount of discharge small.

GEOFFROY SAINT-HILAIRE and CUVIER (16) described a discharge of blood, with enlargement of the sexual organs each month, in *Cercopithecus*, *Macacus*, and *Cynocephalus*—three species of Monkey. [See also EHRENBERG (9) and NUMAN (51)].

Recently J. BLAND SUTTON has paid some attention to the subject, and in a paper published in the 'British Gynæcological Journal' (74) has given an account of his observations.

He finds that *Macacus* menstruates fairly regularly, and that there is a discharge of blood. He states there is no shedding of the lining epithelium of the uterus, and no disintegration of the mucous membrane; the latter, however, becomes congested, and blood emerges therefrom in much the same way as blood escapes from the nasal or buccal cavities in Man, during congestion.

SUTTON'S results, then, differ much from those detailed in the present paper for *S. entellus*. His figures are too diagrammatic to enable one to form a very definite opinion, but it would appear probable he has missed my Stages IV., V., VI., and VII., unless, indeed, the menstrual process in *Macacus* is very much curtailed when the animal is kept in this country.

A cursory examination of the menstruating uteri of *M. rhesus*, which I collected in India, gives me very strong reason to believe that a process, almost identical with that described for *S. entellus*, takes place also in *M. rhesus* during menstruation.

Possibly the cold climate of England acts as a check on the free menstruation of animals which naturally live in a much warmer climate, and this may be the reason why SUTTON, who, I fancy, obtained all his material in London, did not see in them the denudation process.

The above is the only paper I know of in which any attempt is made to describe the histological changes which take place in the uterus of the Monkey during menstruation.

2. *Menstruation in Man.*

I pass now to the phenomena of menstruation as described for the human female. An immense amount of work has been done in this connection. I do not propose to enter here into an exhaustive criticism of the voluminous literature of the subject. From time to time critical accounts have been published, the most recent with which I am acquainted being MEYER'S (46) and STEINHAUS' (70), both of which appeared in 1890.

It appears to me advisable, however, to refer briefly to the work of some of the more recent authors, and to point out where their results and the results arrived at in the present paper differ or are in accord.

There are few observers who now hold the view that no denudation takes place during menstruation; most of them consider that, at any rate, the epithelium, or part

of it, is cast off, and many have come to the conclusion that, although WILLIAMS (78, 79, and 80) has possibly exaggerated the extent of the denudation, yet still a certain portion of the stroma is expelled as well as the epithelium of the mucosa.

It does not seem to be improbable that the extent of the denudation normally varies in different individuals, and, further, that the same individual experiences more or less severe menstrual denudation at different times. This variation, in the severity of the process, may possibly account for much of the difference of opinion found amongst gynæcologists on this matter; but a more probable cause of the various views held appears to me to be the fact, that a sufficient number of specimens of human menstruating uteri, in good preservation, are rarely obtainable by any one investigator. For instance, it is not unfrequently stated that uteri at the height of the menstrual period, and filled with blood, are found to be possessed of uterine epithelium intact, and such evidence is considered sufficient to prove that neither the epithelium nor the underlying stroma is cast off during the process. The evidence I have brought forward in this paper, however, shows that, although it is quite true the uterus may be filled with blood while the epithelium is apparently intact—the blood being derived from the lacunæ through minute ruptures in the ˙epithelial covering—examinations of later stages do show denudation, and prove that the full height of the menstrual cycle is not reached when the blood first flows into the uterine cavity.

DE SINÉTY (68) examined the uteri of many women during different stages of menstruation, and he asserts that in no case was desquamation observed, and that the epithelium was always present. DE SINÉTY explains that the specimens he examined were taken from women who had died from severe cold, and were practically frozen : he claims that, on this account, his material was in a better state of preserva‧ tion than could possibly be obtained in any other manner, and decides that when the uterine epithelium is shed it is due to pathological conditions.

It appears to me more than probable that the severe cold which caused the death of the women, also arrested the progress of menstruation, and that the uteri he examined were in a state of suppressed menstruation; if this is so, his results would be sufficiently explained.

The suppression of menstruation in consequence of a chill is a matter of very frequent occurrence, how much more then would cold which was sufficient to cause the death of the subject, serve to arrest the progress of a function which may be so very readily disarranged.

An extraordinary theory, broached by Dr. HOTTENIER, and drawn attention to by DE SINÉTY, is founded on these results. It claims that, as the menstrual blood cannot be shed between the epithelial cells, it follows that the glands are the channels through which the blood escapes ; there appears, however, to be absolutely no evidence in favour of this view.

The work of KUNDRAT and ENGELMANN (34), reproduced in an article by the

latter author (11), is perhaps the most important contribution that favours the view that no denudation takes place; these authors state there is no shedding of mucous membrane; cells, however, were found among the débris of the menstrual flow, presumably epithelial cells, but it is denied that the whole of the epithelium is cast off.

A swelling of the mucosa is described by these authors, due to the growth of the stroma of the upper part of the mucosa, also an increase of intercellular substance, and an enlargement of glands and vessels, followed by hæmorrhage.   The hæmorrhage, it is claimed, is always confined to the surface of the lining membrane, and is caused by the rupture of the vessels in that region; this rupture of vessels being due to disintegration changes (fatty degeneration) which is specially marked upon the surface of the uterus.   It is concluded that the hæmorrhage is not due to congestion of the organ, because far greater hyperæmia exists in pregnant uteri without any hæmorrhage.

Much of this work is very much in accord with my own results, but appears to stop short at my Stage IV. The period of growth is fully described, but the period of degeneration is not completed, the swelling of the stroma, increase of vessels, their congestion and hæmorrhage due to hypertrophy and degenerative causes (not fatty degeneration, however) being described in the account I have given above, for *S. entellus.*

I fully agree with these authors that the rupture of the vessels is ultimately due to degeneration, but cannot go so far as to say it is not primarily due to congestion; hyperæmia undoubtedly precedes the rupture of the vessels, and there seems to be good reason to suppose that a state of congestion is naturally followed by degeneration of the walls of the vessels and the surrounding tissue.   The fact that increased hyperæmia exists in pregnant uteri without hæmorrhage, may be readily accounted for by the presence of the embryonic membranes over the surface of the uterus.

MÖRICKE (50) is more emphatic in his statement that the mucous membrane is neither partly nor wholly cast off during menstruation. He says that the interglandular stroma is not increased, and that fatty degeneration, except perhaps to a trifling degree, is never shown. He also describes an increase in the homogeneous ground substance of the mucosa, and states that the vessels enlarge, become filled with blood, and there is an occurrence of extravasation of blood in the upper layer of the mucosa.

This author, then, does not appear to distinguish a period of growth of the same extent as I have described, and denies the existence of a period of degeneration altogether.

More recently OLIVER (52) has expressed somewhat similar views, and states that he has examined uteri *(post-mortem)* both before and during the menstrual period, and has never found evidence of any change in the tissue. So that here we find even a period of growth denied.

It is impossible to reconcile these results with my own researches or with those of the authors quoted below, and one can only suppose, either that the specimens examined were uteri of individuals who were all suffering from suppressed menstruation, a very improbable supposition, or that these authors have not been fortunate enough to obtain a complete series of menstruating uteri.

AVELING (1) urges the view that there is a periodical formation of a membrane lining the body of the uterus, the development taking place during the intermenstrual period ; that in the absence of a fertilized ovum, degeneration, caused by a cessation of nutrition, loosens its attachments, and it is expelled by contractions of the uterus, generally in small portions, but sometimes whole, as a three-cornered bag.

Finally, he believes that menstruation is probably determined by the act of "denidation," as he calls it, because it is from the denided surface that the menstrual flow comes.

This author considers the formation of the membrane and menstruation as two separate processes, the former being a preparation of the uterus for the reception of an ovum, while the latter is a secondary process due to denidation.

I will refer to this view later on, and will here merely remark that my own results are to some extent in accord with those of AVELING. He finds and differentiates periods of growth and degeneration as I do, but he appears to consider them less closely allied than my observations allow me to admit.

WILLIAMS (78, 79, and 80) describes a period of growth in the mucous membrane prior to menstruation, and describes the latter as produced by fatty degeneration of the uterine mucosa. This degeneration, he says, commences within the internal os and extends to the fundus, is followed by disintegration ; which includes the whole of the mucous membrane, the glandular and mucous elements, and the walls of the superficial vessels ; and thus causes hæmorrhage. In this author's opinion the denudation is so complete that the muscular coat is laid bare. The re-formation of the mucous membrane is described as beginning at the internal os and extending gradually towards the fundus, the new tissue being produced partly from the fibres of the muscular coat and partly from the stroma.

WILLIAMS' results have not been generally accepted ; it has been argued that his material, mostly derived from subjects who died from fever, was unsatisfactory, and that doubtless the completeness of the denudation was due to disintegrating changes produced, not by menstruation, but by the disease from which the patient suffered. Exception has also been taken to his description of the re-formation of the mucosa from the muscle layer.

WILLIAMS has attempted to meet this latter objection (79) by arguing that the inner muscle layer of the human uterus is really a portion of the mucosa, and he has presented a comparative account of the structure of the uterus in various animals in support of his view.

I have not myself had an opportunity of investigating the comparative anatomy of

the uterus, nor, what is more important, the comparative development of the mucosa, and cannot offer an opinion on the relation of the muscular layer of the human uterus, I can only say that in *S. entellus* the denudation is superficial and does not approach the internal muscle layer.

I do not agree with WILLIAMS in ascribing the degeneration to fatty degeneration, but that is a small point ; our results agree as regards the occurrence of periods of growth, degeneration, and recuperation; but WILLIAMS denies there is any period of rest, and states the nearest approach to uterine rest is during the menstrual flow when disintegration is going on.

Here, again, our results differ ; I find that the epithelium begins to re-form while the menstrual flow is still going on, and that there is certainly no sign of rest at that stage.

WILLIAMS appears to think the process of growth in the mucosa occupies the whole of the time after the cessation of the menstrual flow until degeneration again occurs ; whereas I find there is a space of time after the flow has stopped, and after the recuperation has concluded, during which the tissue of the mucosa is in a state of rest.

UNDERHILL (77) finds in the uterus of a woman who died immediately after menstruation, that the superficial part of the mucosa was wanting, but he disagrees with WILLIAMS in stating that all the mucous layer is shed.

LEOPOLD's researches (38) lead that author to express the view that pieces of the superficial mucosa are cast off, and that the bleeding is derived from the capillaries, which are much swollen, by diapedesis ; and he denies that bleeding is due to fatty degeneration. He describes a growth of the mucosa which is so considerably that it almost entirely fills up the cavity of the uterus, prior to the bleeding, and a subsequent return to its normal thickness.

The regeneration of the mucous membrane, he states, begins at the close of the bleeding stage. The swollen vessels of the upper surface are partly resorbed, partly broken off, and the gaps left in the superficial mucosa by denudation are filled up by the growth of neighbouring cells, and the cylindrical epithelium of the glands. The author gives a description of the histological elements of the human mucosa which is very different from that which obtains in the mucosa of *S. entellus*.

I cannot but think it will be found that LEOPOLD's results are based upon an incomplete series of menstruating uteri.

WYDER (82, 83) believes there is a variable amount of the upper part of the mucosa cast off in different cases, sometimes the whole of it being denuded, at other times the amount is minimal (SPIEGLEBERG (69)). In all cases, however, a well preserved portion is left behind. The denudation he considers due to menstrual bleeding and not to fatty degeneration, the latter, in his opinion, being a consequence of the destruction and denudation of the tissue after the bleeding. The regeneration of the

epithelium he describes as due, as much to the glandular as it is to the remaining epithelial cells of the surface of the uterus, which remain behind.

WYDER calls attention to the marked difference between the cells of the upper and middle layer of the mucosa of the menstruating uterus and the cells of the decidua of a pregnant uterus, and suggests the cast-off portion should be called mucosa menstrualis instead of decidua menstrualis, in order to avoid confusion. This suggestion I have adopted in the account given of my own observations. I cannot agree with this author that degeneration of the mucosa is due to the denudation process; in my preparations degenerated tissue is undoubtedly present before denudation takes place.

JOHNSTONE (29) describes the mucosa of the menstruating uterus as similar to that of the non-menstruating organ, except that the epithelium is cast off and washed away. He considers the material used by WILLIAMS to be unsatisfactory, and criticises AVELING'S observations as entirely erroneous. He believes the growth of the corpuscular element of the mucosa ("endometrium") is continually going on by means of the gradual growth of the granular elements contained in the "sustentacular threads," and not by division of the cells themselves; and that the products of this growth in the non-pregnant uterus are washed away by the menstrual flow. I cannot discover that JOHNSTONE presents sufficient evidence to render his views of the formation of cells *de novo* at all probable: and his description of the "continual" growth of the mucosa is probably due to the fact that he has not seen menstruating uteri throughout the whole of the denudation period.

OVERLACH (53) finds the upper surface of the mucosa is almost entirely cast off. He considers the cause of menstrual bleeding to be venous congestion, brought about by the compression of the veins in the muscular wall of the uterus, and the actual escape of blood to be derived from the capillaries within the mucosa by diapedesis, and from those on the surface by rupture. In contradistinction to WYDER, OVERLACH believes that a formation of decidual tissue does take place. (See also GUSSEROW (19), and LEVY (40)).

FEOKTISTOW (13) believes there is no doubt that fatty degeneration may appear in the menstruating mucosa, and that there may be a loss of uterine epithelium, but the menstrual bleeding he considers an inconstant and immaterial symptom.

KAHLDEN (30) says that in all probability the whole of the epithelium of the surface, together with a considerable part of the mucous membrane, is cast off during menstruation; a conclusion with which LÖHLEIN (41) does not agree.

Thus it is seen there are a great variety of views held by modern investigators of the process of menstruation in the human female, as to the actual phenomena which occur, almost as many views indeed as there are writers on the subject.

The extreme view on the one hand that there is no change in the tissue during menstruation is opposed by the extreme view on the other hand that highly specialized decidual tissue is formed in the mucosa at that time. Again, the statement that not even a portion of the epithelium is lost by denudation is opposed by

the statement that the whole of the epithelium, together with all the underlying mucous membrane, is discarded during menstruation.

To reconcile such diametrically opposite views is impossible. It is no doubt possible that all these extreme cases may have occurred, though, in my opinion, that is doubtful ; but it is quite certain that neither the one nor the other is descriptive of the phenomena which normally occur in menstruating uteri. The majority of writers appear to hold that a growth of the stroma takes place, and that it is followed by more or less complete denudation of the epithelium and the superficial stroma ; that the denudation is preceded by or accompanied by bleeding from the congested capillaries in the superficial mucosa, either by diapedesis or by rupture of the vessels, or by both processes ; that more or less degeneration of the tissue of that region occurs, and that the denudation is due either to the extravasation of blood, or to the degeneration of the tissue.

These views are, in a general way, similar to those advanced by me for *S. entellus* ; the details are very variably described however, and only extended researches can satisfactorily determine which are correct.

The difficulty experienced by any one investigator in obtaining a sufficient number of specimens of human uteri, and the rare opportunities which present themselves of getting healthy uteri immediately after death, have no doubt been the main causes of the different descriptions given, and the diversity of views held, of the changes which take place during menstruation ; but it is also very possible that different individuals experience more or less severe menstrual periods, or that the same individual experiences more or less severe periods at different times.

In comparing the results arrived at by my investigations on *S. entellus* with the records of writers on menstruation in the human female, it should be remembered that I was fortunate enough to get an ample supply of well-preserved material and that the material was derived from healthy females, killed for the purpose.

This being so I have some confidence in my belief that the various phases represented in my figures are not abnormal phases, though I cannot assert they invariably occur in the same individual, or in another individual to the same extent. As a matter of fact, however, I have in no case relied upon a single specimen to prove the existence of any one stage ; I have always had three or four, and generally more, specimens referable to the same stage.

3. *The Period of " Heat " in Animals.*

A comprehensive account of the period of " heat " in animals has still to be written. I do not attempt here to present an adequate account of what is known of the subject, but some interesting work has been done recently which has no little interest to students of gynæcology, and requires mention here.

Bonnet (3 and 4) describes the formation of pigment in the uterine mucous membrane of the Sheep from extravasated blood-corpuscles which he says are first

seen in the deeper part of the layer and are subsequently carried to the surface by wandering cells.

KASSANDER (31) finds scattered blood-corpuscles lying in the midst of the tissue of the uterine mucous membrane of the Sheep in an early stage of hæmorrhage ; he states they rarely occur in the deeper tissue but they are seen in numbers near the surface; he denies the existence of the wandering cells described by BONNET, but agrees that the corpuscles give rise to pigment in the tissue.

ELLENBERGER (10) describes congestion of the sexual organs and secretion from their glands during "heat," he states also the mucous membrane of the uterus is swollen and reddened, and a little bleeding takes place on the surface of the organ. In Ruminants the bleeding occurs at the cotyledons.

BONNET in an article in the same work (5) confirms ELLENBERGER and adds, the bleeding may occur within the mucosa of the uterus.

RETTERER (63) again confirms ELLENBERGER'S statement of the modification of the mucous membrane, and describes other changes which take place in the mucosa of the Bitch during "heat," which are of great interest.

The capillaries, it seems, which are rare and small when the uterus is in a quiescent state, become during "heat" much more numerous and larger; their dilatation results in rupture in the superficial part of what he calls the "chorion," in which numerous spots of extravasated blood are formed. Blood, he adds, is poured into the cavity of the uterus, but he does not believe the uterine epithelium is shed. He finds the epithelium is less firmly attached to the "chorion" during "heat," and that it is absent here and there, but he attributes its disappearance to faulty manipulation. Finally he describes hypertrophy of the "chorion" during "heat," and shows that pigment is formed in the hæmorrhage centres from masses of red blood-corpuscles.

These observations of RETTERER'S are very interesting and do much to prove that menstruation and "heat" are processes very closely allied. They show that the mucosa of the Bitch undergoes changes probably (in the absence of figures it is not possible to say more) very similar to those described in the present paper from Stage I. to Stage V. or even VI., that is, as far as the formation of lacunæ (RETTERER'S "spots"), and probably as far as the escape of blood from the lacunæ into the uterine cavity. The denudation stage is not represented in the Dog according to RETTERER, although it seems to me very probable that the loss of epithelium, which he thinks due to faulty manipulation, may well be caused by denudation. This view is rendered all the more probable because the epithelium is described as less firmly attached to the "chorion" during "heat."

We may say then, that a period of growth followed by a period of degeneration occurs in the Dog during "heat," probably in much the same way as they occur in menstruation, except that the degenerative changes are not carried so far.

4. *Ovulation in the Human Female and other Animals.*

On the subject of ovulation in the human female, STEINHAUS (70) gives a very excellent *résumé* of the literature, and concludes that the evidence brought forward shows, in the first place, that ovulation in Man is a spontaneous occurrence, in so far as it is due to internal energy without the concurrence of provocation from the exterior; and in the second place that ovulation is not periodic, and does not necessarily occur in connection with menstruation.

REEVES JACKSON (59), who also gives a critical account of previous writers, concludes that ovulation and menstruation may occur independently, that ovulation is an irregular but constant function of the ovaries, while menstruation is a rhythmical function of the uterus. He considers that the maturation and rupture of the Graafian vesicles is not necessarily connected with menstruation, and that menstruation may persist after the removal of the ovaries. AVELING (1) says it seems certain that ova are discharged from the ovaries at irregular periods, and not once a month at or near the menstrual period. LAWSON TAIT (37) also believes that menstruation and ovulation are not concurrent; and a similar conclusion is come to by RAYMOND (58).

GUSSEROW (19) and LEVY (40), on the other hand, believe ovulation and menstruation are coincident; WILLIAMS (81) that they are closely connected, while LEOPOLD (38 and 39), after closely investigating the material at his command, considers there is not sufficient evidence to determine the question with certainty.

LÖWENTHAL (42) holds that the condition of bleeding of the uterus induces a force which acts as a cause for the bursting of a ripe follicle; but he considers the menstrual bleeding may appear without the simultaneous bursting of a follicle. Finally, SANDERS (64) seems to think that coitus is an excitable cause of ovulation.

The conclusion, that the majority of recent writers are in favour of the view that ovulation is not necessarily coincident with menstruation, is in harmony with the result at which I arrived after examining the ovaries of *S. entellus* in connection with menstruation; further, my suggestion that the increased blood supply to the generative organs during menstruation may induce ovulation when a sufficiently ripe ovum is present, receives the support of LÖWENTHAL'S observations.

The method adopted to determine the question of the connection between ovulation and menstruation is beset with much difficulty. It is desired to know the age of corpora lutea, and the condition of ripeness of prominent Graafian vesicles. In the first place I greatly doubt the possibility of determining accurately when a Graafian vesicle is perfectly ripe, and in the second place I doubt if it is possible to be assured of the exact age of a "false" corpus luteum.

LEOPOLD in his earlier paper makes allusions to corpora lutea one to two days old, three weeks old, or four weeks old; and states with regard to the Graafian vesicle of a girl who died just before menstruation, that if the girl had lived the follicle would have burst the next day; and WILLIAMS makes assumptions somewhat similar.

I would suggest that such statements can only be made if it is already assumed that the process of ovulation takes place at certain known times, only then, with the knowledge at present at our command, can the age of a corpus luteum or the date of the bursting of a follicle be determined. To assume this knowledge is, however, to presume acquaintance with the problem it is desired to solve, and I venture to think that results founded on this method are not trustworthy.

Ovulation in the lower Mammals is generally, if not universally, considered to be coincident with the period of "heat." RETTERER (63) says this is so in the Dog. He draws attention to the fact that if the ovum of a Fowl be prevented from entering the oviduct, albumen is still secreted by the latter, and round the albumen a shell is formed and it is laid. The author thinks the congestion of the uterus of the Dog is due to an analogous mechanism, and appears to assume that ovulation produces menstruation in Woman, and that "heat" and menstruation are analogous processes.

In the lower animals then, ovulation and "heat" are said to be coincident, but whether the latter induces the former, or the former the latter, there is no evidence to show.

5. *Some Theories of the Cause and Function of Menstruation and "Heat."*

With regard to the cause of the hæmorrhage :—AVELING (1) considers "denidation" is caused by a cessation of nutrition. KUNDRAT and ENGELMANN (34) go farther and urge the view that fatty degeneration is the cause of menstrual bleeding, an opinion shared by WILLIAMS (78) and opposed by WYDER (83). GOODMAN (17) holds that congestion is the principal factor in, if not the sole cause of hæmorrhage, and that congestion is produced by the contraction of involuntary muscles round the vessels. OVERLACH (53) also thinks congestion, caused by compression of veins in the muscle coat of the uterus, is the cause of menstrual bleeding.

JACOBI (28) suggests that the mechanical effects of pressure caused by the growth of the opposite walls of the uterus against each other, described also by LEOPOLD (38), is sufficient to bring about rupture.

Although I find congestion in the superficial vessels, I have no evidence of any compression of the veins in the muscular coat which would cause congestion, and HELME's work (21) appears to be unfavourable to such a view ; also I cannot agree with JACOBI's suggestion of the mechanical effect of pressure, there is no evidence whatever that any pressure is exerted in the case of *S. entellus*, the cavity of the uterus is not obliterated at any stage of menstruation, until the menstrual clot is formed and the period of recuperation sets in, and yet very extensive bleeding occurs in this animal. My own preparations show that hyperplasia of the vessels followed by congestion and degeneration is the immediate cause of the extravasation of blood and subsequent menstrual hæmorrhage.

The primary cause of menstruation remains unexplained ; the old view that ovula-

tion is the cause of menstruation is, in my opinion, no longer tenable. OLIVER (52) considers menstruation has a nervous origin.

The function of menstruation seems more capable of explanation. AVELING (1) broadly states it is a primary reproductive function. GEDDES and THOMPSON (15) that it is a means of getting rid of anabolic surplus in the absence of the consumption thereof by an embryo.

KUNDRAT (KUNDRAT and ENGELMANN (34)) more particularly defines menstruation as designed to prepare the uterus for the reception of an ovum, while his fellow author, ENGELMANN (11), does not share that view. LAWSON TAIT (37) also considers that menstruation is in reality a preparation of the surface of the mucosa for the retention of an ovum, while at the same time he draws attention to the fact that pregnancy is possible without menstruation. LÖWENTHAL (42) goes so far as to propound the belief that the menstrual "decidua" is actually produced by the embedding therein of an unfructified ovum, and that the pregnancy decidua is built up if the ovum is fertilized, whereas, if it is not fertilized, the decidua falls to pieces. He brings forward no evidence which, in my opinion, supports the first part of his theory.

JOHNSTONE (29) considers that the design of menstruation is to change the uterine mucosa, a view also held by FEOKTISTOW (13) and others.

In strong contrast with these views is that of KING (32). He considers menstruation is a disease, an abnormal, unnatural, acquired habit, due to the fact that Women do not breed early enough. The fact that vessels rupture during menstruation, he argues, is proof that the process is an unnatural one, and in support of his argument he urges that abnormal congestion in organs gives rise to fibroid growth, and that fibroid growths in the uterus are common.

The evidence which KING brings in support of his theory is criticized by STUDLEY (73), and shown to be capable of other interpretation, while the work of RETTERER (63) on the Bitch, my own researches on *S. entellus*, and KRIEGER (33), TILT (75), RACI-BORSKI (57), PLOSS (55), VON ICARD (26), and many others on the human female, may be quoted as directly opposed thereto.

Finally, PLAYFAIR (54) says the purpose of the loss of blood in menstruation is quite unknown.

The view that menstruation is designed to prepare the uterus for the reception of an ovum, seems to be the view most generally maintained; it is held by those who believe denudation takes place and by those who believe no denudation occurs. The one presumably believes it is the growth of tissue which prepares the uterus for the reception of the ovum, the other that it is the changing of the tissue by denudation, which is the important phase of the preparation. These views are, therefore, contradictory, and, doubtless, the contradiction is due to the fact that the period of growth and the period of degeneration have not been clearly recognized. For my part, I consider there is strong reason to think that the growth of the stroma is, in fact, a

preparation of the uterus for the reception and retention of an ovum, and that the subsequent degeneration, bleeding, and denudation are due to the absence of a fertilized ovum in the uterus at that time.

6. *The Connection between "Heat" and Menstruation.*

REEVES JACKSON (59) and LAWSON TAIT (37) deny that "heat" and menstruation are homologous, while PLAYFAIR (54) considers they are probably analogous functions. RETTERER (63), who believes in the ovulation theory of menstruation, considers "heat" and menstruation are analogous ; and certainly his work on the Bitch, and mine on *S. entellus*, show there is a marked similarity between the histological processes which occur during the period of "heat" and menstruation of these animals. This similarity is especially emphasized in regard to the period of growth, and I am much inclined to think it will be found that growth of the mucosa universally occurs in the uterus of animals "on heat." I have observed in the uterus of the Mole at breeding time and prior to fertilization that the mucosa is swollen and tumid ; the same appearance is also present in the uterus of the Rabbit when "on heat" ; and PLAYFAIR (54) says the mucous membrane of the human uterus becomes thickened and vascular before the ovum reaches the uterus.

The relation of "heat" to fertility, the accompanying desire for sexual intercourse, a desire which is absent in the females of the lower Mammals at all other times, and the coincidence of "heat" and ovulation, make it not surprising to find that this growth of the mucosa is recognized as one of the earliest phenomena attending the formation of the placenta. I have observed that this is so in the Mole (see also STRAHL'S figures (72)) ; MINOT (47) and MASQUELIN and SWAEN (43) find it in the Rabbit, and HEIN-RICIUS (20) in the Dog.

So that in these animals, whatever may be the cause of this growth of the mucosa during "heat," its probable function is the preparation of the uterus for the reception of fertilised ova, which the coincidence of ovulation and "heat" enables the uterus to anticipate with some certainty.

I think it may be assumed that the majority of females "on heat" in the wild state are impregnated whenever their condition renders copulation possible, and this being so, it is not surprising to find the uterus anticipating the arrival of fertilised ova. In human beings, however, these relations no longer exist ; menstruation is not coincident with ovulation nor does a desire for sexual intercourse prevail during menstruation ; further, ERCOLANI (12) states that the growth of tissue to form the placenta in the human female is quite distinct from that of other animals ; but in view of the most interesting researches of HUBRECHT (25) on the Hedgehog this statement requires confirmation.

I conclude then, that the similarity of the histological processes which occur in "heat" and menstruation is enough to show that they are analogous processes, and I believe

that the differences which exist are referable to the increased complexity of the phenomena attending breeding in the higher animals.

I do not know whether Monkeys copulate during menstruation or not; that they have a special breeding season or seasons appears certain, but whether sexual intercourse is admitted at other times or not, is not, as far as I am aware, known.

In connection with this subject the nervous relation between the flushed area on the buttocks, thighs, and tail of *M. rhesus* and the vagina is of interest. The joint researches of LANGLEY and SHERRINGTON (36) and SHERRINGTON (66 and 67) show that the motor roots of the Ist, IInd, and IIIrd sacral nerves supply the vagina, while the sensory roots of these nerves supply the flushed area described above. This area is always more or less flushed in *M. rhesus*, but is specially noticeable in the female during menstruation and pregnancy, when it is very highly congested, and would undoubtedly appear to be influenced by sexual phenomena.

The uterus of the Rabbit, however, according to LANGLEY'S (35) observations, receives motor fibres from the sympathetic chain, from about the IVth to the VIth lumbar ganglia, and is not certainly affected by stimulation of the sacral nerves.

I have mentioned these facts because the swelling which is observed round the external generative organs of animals during the season of "heat," is probably homologous with the enormously swollen area exhibited by certain Baboons during menstruation, and with some portion, at any rate, of the flushed area of *M. rhesus* at this time, and it is of interest to know that there is a nervous connection between these parts and the vagina.

A further examination of the physiological relation between the vagina and the uterus would probably throw much light upon the origin of menstruation and "heat."

In conclusion, it appears to me that sufficient stress has not been laid upon the different periods of the menstrual process, and that a consideration of them, although it does not explain the origin, leads to an explanation of the function of menstruation.

The period of growth of the stroma is the primary phenomena attending menstruation. I cannot admit that AVELING (1) is right to separate the growth of the stroma from what he calls the "denidation." This growth of tissue is closely bound up, indeed is indissolubly connected, with the phenomena which subsequently appear; it is the primary phase of menstruation, and I am convinced it is to a determination of the cause or causes regulating and inciting this growth we must look for an explanation of the origin of the process.

NOTE.

Since finishing this paper, I have seen MARSHALL'S recent book on 'Vertebrate Embryology' (42A). In it he divides menstruation into four stages identical with the four periods into which I have divided the process in the present paper.

MARSHALL is also of opinion that the period of growth is in effect a preparation of the uterus for the reception of an embryo, but he goes further and seeks to show that

the degeneration stage is not to be regarded as an undoing of the preparation made during the period of growth, but as a further continuance in a modified form of the act of preparation, leaving the uterus in a condition in which, for further elaboration to occur, the presence of an embryo is necessary.

I do not understand from this account whether MARSHALL believes that the uterus is in a condition to receive an ovum during the period of degeneration, or whether, after the recuperation period is over, the uterus is in a condition in which, for further elaboration to occur, the presence of an embryo is necessary.

Neither of these views, however, appear to me tenable.

With regard to the first view, a glance at my fig. 8 will, I think, show that it is exceedingly improbable an embryo would be retained in the uterus in the face of the active denuding process which is there going on; and, in regard to the second view, its adoption seems to me to entail the rejection of his previous assertion that the period of growth is in effect a preparation of the uterus for the reception of an embryo.

It is true that ERCOLANI (12), MINOT (47), and others have shown that degenerative changes do occur in the mucosa during the formation of the placenta soon after the embryo becomes attached to the maternal tissue, but, as far as I can understand, the changes are due to absorption by the embryo, and are not in any way similar to the degeneration which precedes and accompanies denudation.

SUMMARY.

The phenomena of menstruation are grouped into four periods which are subdivided into eight stages.

A. Period of Rest.
    Stage I. The resting stage.
B. Period of Growth.
    Stage II. The growth of stroma.
    Stage III. The increase of vessels.
C. Period of Degeneration.
    Stage IV. The breaking down of vessels.
    Stage V. The formation of lacunæ.
    Stage VI. The rupture of lacunæ.
    Stage VII. The formation of the menstrual clot.
D. Period of Recuperation.
    Stage VIII. The recuperation stage.

Superficial Phenomena of Menstruation.

External.—A swelling of the labia and of the nipples takes place, and a discharge from the vagina, consisting of mucus, leucocytes, blood, stroma, and epithelial cells.

· Internal. Stages I. and II.—Mucosa an opaque white colour, either smooth or swollen into folds, ridges, or polygonal areas by the growth of the stroma during Stage II.

Stages III. and IV.—Mucosa uniformly flushed when smooth, but when folded the flush is concentrated on the edge of the ridges. The flushing is exaggerated during Stage IV.

Stage V.—Dark red spots are seen on the mucosa—lacunæ. The increased supply of blood affects the dorsal before it affects the ventral wall of the uterus.

Stage VI.—Free blood is found in the cavity of the uterus, and is due to the rupture of the lacunæ.

Stage VII.—Shows the formation of the menstrual clot, which consists of red blood corpuscles, leucocytes, epithelial cells, and stroma.

Stage VIII.--The mucosa appears at first with a ragged surface, and then with a smooth one, the latter stage being due to the growth of the epithelium. The menstrual clot is still in the uterus at the early part of this stage, at the latter part the mucosa has a transparent appearance.

Histology of Menstruation.

General.—The body of the uterus consists of mucosa and muscle layers.

The mucosa is formed of uterine and glandular epithelium, a primitive tissue consisting of a network of protoplasm in which nuclei are embedded and which I have called stroma, blood vessels, and a few radial muscles.

The internal muscle layer is composed chiefly of circular bundles with fewer longitudinal bundles ; the external muscle layer chiefly of longitudinal bundles with fewer circular bundles amongst them. The sheath is a thin layer of scattered circular and longitudinal muscle fibres, together with a few connective tissue cells, and is covered by a flat epithelium.

In the cervix, the mucosa gives place gradually to a layer of denser material, the cylindrical epithelium of which is not cast off during menstruation. The walls of the Fallopian tubes carry a continuation of the uterine mucosa as a thin layer of stroma surmounted by cylindrical epithelium. There is no change of structure in the tubes during menstruation.

A. Period of Rest.

Stage I.—The uterine epithelium is a single row of cubical or columnar cells, containing round nuclei, the outer edge of the epithelium is sharply defined in section, the protoplasm of the inner edge is continuous with the protoplasm of the stroma network below. The uterine epithelium is continuous with that of the glands, the latter rest on a basement membrane but have no sheath. The stroma

has round nuclei embedded in a continuous network of protoplasm, the processes are very fine and delicate.

For one-third of the depth of the mucosa the stroma is regularly disposed, below that, fibrils run through it fan-wise, they are formed of processes of the stroma joined together. The blood vessels are small, but fairly numerous.

B. *Period of Growth.*

Stage II.—An increase in the number of stroma nuclei by amitotic division, and probably fragmentation, causes swelling and increase of the density of the upper third of the mucosa—hyperplasia. Owing to pressure the nuclei become fusiform. In the deeper layer there is no change in the stroma. An enlargement of vessels in the deeper mucosa follows the growth of the stroma in the upper part. The swelling takes place in the interglandular regions. The epithelium of the uterus and glands is very little altered.

Stage III.—The dense layer of nuclei of the last stage is rendered less dense on account of the swelling of the stroma and the stretching of the epithelium covering it. Hyperplasia of the vessels takes place below the epithelium, giving rise to the flush seen on the surface.

The swelling of the stroma causes an increase in the thickness of the mucosa and a widening of the glands. The hyperplasia of the vessels is a natural result of hyperplasia of the stroma. The size of many of the stroma nuclei is reduced.

C. *Period of Degeneration.*

Stage IV.—Simple hypertrophy of the uterine epithelium, stroma, and walls of the vessels now appears all over the mucosa, followed by degeneration in the superficial region where the dilated, congested capillaries break down, and the blood contained therein is extravasated. I see no signs of fatty degeneration, and am inclined to consider the degeneration observed to be of the amyloid or hyaline type. There is a decided increase in the number of leucocytes, which travel to the surface of the mucosa by the blood vessels, collecting there in the dilated capillaries.

There is no sign of migration of leucocytes or diapedesis of red blood corpuscles, but where vessels are ruptured, leucocytes are swept out, together with red blood corpuscles, into the surrounding stroma tissue. The congregation of leucocytes in the dilated capillaries near the surface probably indicates the occurrence there of an ʿinflammatory substance.

Stage V.—The extravasated blood collects into lacunæ, which are first formed within the stroma, but gradually extend themselves superficially, displace the intervening stroma tissue, and come to lie directly below the epithelium. All superficial dilated capillaries now break down, but vessels in the deeper mucosa remain intact. There is no trace of leucocytes or red blood corpuscles in the tissue of the deeper mucosa.

Certain stroma cells and free leucocytes now appear to be undergoing degeneration.

Stage VI.—The uterine epithelium and superficial stroma now shrivel up and become degenerate. The lacunæ increase greatly in size, and, in consequence of the rupture of the degenerated uterine epithelium covering them, the blood contained within them is poured into the uterine cavity.

The lacunæ are generally in the neighbourhood of glands, and sometimes they entirely surround a gland, so that when rupture takes place the whole gland is washed away. Large numbers of leucocytes are seen, for the most part within vessels, and, if the latter are ruptured, the leucocytes seem to stick to the remains of their walls rather than to migrate into the tissue.

Vigorous nuclear reproduction takes place in the leucocytes, but no division of the leucocyte cell was seen. It is probable the increased number of leucocytes is due to an increased supply rather than to reproduction *in situ.*

Stage VII.—Denudation now takes place, and is very complete; all the uterine epithelium, a portion of the glands, and in some places a whole gland, and a portion of the stroma layer are torn away, together with ruptured vessels, red blood corpuscles, and leucocytes—a severe, devastating, periodic action which is very remarkable. Much of the mucosa menstrualis consists of shrivelled degenerated cells, but there are many normal cells amongst them. A ragged surface is left behind, and the remaining stroma contains masses of extravasated blood. In the deeper parts of the mucosa there is no further change in the tissue.

D. *Period of Recuperation.*

Stage VIII.—The recuperation consists of the re-formation of the epithelium, partly from the torn edges of the glands and partly by means of the transformation of stroma elements into flat epithelium cells. Gradually the flat cells become more cubical. There is no pus formed on the wounded surface. There is a cessation of the blood flow, probably owing to uterine contractions. New capillaries are formed superficially from stroma cells surrounding intercellular spaces in which blood corpuscles lie. Great activity is exhibited in enclosing all the extravasated blood corpuscles which remain in the tissue, and in returning them to the circulatory system. These capillaries eventually disappear. There is a recuperation of the vessels in the deeper mucosa; they return to their normal size and consistency.

Further, there is a return of the stroma to its condition of rest, accompanied by a limited amount of multiplication of nuclei by amitotic division, and probably also fragmentation. A general shrinkage of the mucosa takes place, the stroma first retiring, the epithelium following, the cells of the latter becoming columnar in the process, and folds in that layer being formed which give rise to new glands.

The leucocytes which were left with the extravasated blood in the tissue are returned to the circulatory system by means of the new vessels; they do not form new tissue *in situ,* nor migrate, and seem to have been induced to appear on

the scene, in such large numbers, unnecessarily; the casting away of the menstrual mucosa, together with all noxious material, and the clean healing of the wounded surface, rendering their protective presence unnecessary.

Ovulation.—There appears to be sufficient proof that ovulation is neither the cause nor the necessary result of menstruation. It is possible, however, that the increased blood supply to the generative organs during menstruation may induce ovulation when a sufficiently ripe ovum is present.

Account of Recent Literature and Conclusions.

1. *The Menstruation in Monkeys.*—The only paper on the history of this subject is by SUTTON, who describes a discharge of blood into the uterus, but denies that any denudation takes place in *M. rhesus.* I have got menstruating uteri of *M. rhesus,* however, in which denudation is shown.

2. *The Menstruation in Man.*—On this subject a great variety of opinions are held. The majority of authors, however, hold that growth of the tissue of the mucosa takes place, followed by more or less denudation, and accompanied by bleeding from congested capillaries, either by diapedesis or rupture, or both processes; that degeneration occurs, and that denudation is due either to degeneration of tissue or extravasation of blood. These views are very similar to those advanced for *S. entellus* in this paper. The variety of views on human menstrual phenomena is probably due to the difficulty of obtaining a complete series of healthy uteri properly preserved.

3. *The Period of "Heat" in Animals.*—RETTERER's observations show that the mucosa of the Bitch during "heat" undergoes changes probably very similar to those described for *S. entellus* from Stages I. to VI., the denudation not being represented according to this author, although there is an escape of blood. Periods of growth and degeneration, therefore, both occur in the mucosa of the Bitch during "heat."

4. *Ovulation in the Human Female and in other Animals.*—It is found that the majority of writers are in favour of the view that ovulation is not necessarily coincident with menstruation in the human female, a view in harmony with that expressed by me for *S. entellus,* whereas in the lower Mammals ovulation and "heat" appear to be inseparable.

5. *Some Theories of the Cause and Function of Menstruation and "Heat."*—The hæmorrhage during menstruation is chiefly attributed to either congestion or degeneration. The primary cause of menstruation remains unexplained. The function of menstruation is variously represented, but it is largely believed to be a preparation by the uterus for the reception of an ovum. I myself hold that the period of growth is a preparation for the reception and retention of an ovum, and that the subsequent degeneration is due to the fact that a fertilised ovum is not present in the uterus at the time.

6. *The Connection between "Heat" and Menstruation.*—The period of growth is found

alike in the phenomena attending "heat" and menstruation, and in the lower Mammals, at any rate, a similar growth is found in the early development of the placenta. The function of this growth I consider to represent the preparation of the uterus for the reception of an ovum, which the coincidence of "heat" and ovulation enables the uterus in these animals to anticipate with some certainty. The histological similarity of the mucosa during the period of "heat" in the Dog and menstruation in *S. entellus* shows that these processes are analogous, while the differences which exist are, in my opinion, referable to the increased complexity attending the process of breeding in the higher animals.

CONCLUSION.

In the description which has been given of the menstruation of *S. entellus*, attention has been drawn to the primitive nature of the stroma, of which the mucosa is largely composed. The most remarkable changes which take place in the mucosa have been indicated, and some idea given of the part played by the various tissues concerned. An endeavour has been made to establish the fact that the monthly history of the adult non-pregnant uterus consists of four periods, namely, A, rest; B, growth; C, degeneration; and D, recuperation. The existence of these periods, although they cannot be quite sharply defined, are, nevertheless, very marked and real, and they indicate that a substantial periodic growth of the mucosa is arrested by degenerative changes when a fertilised ovum is not present.

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DESCRIPTION OF PLATES.

Reference Letters.

a.	Artery.		*lac.*	Lacuna.
b. m.	Basement membrane.		*leu.*	Leucocyte.
bl. ex.	Extravasated blood in stroma.		*mc.*	Mucosa.
bl. fr.	Free blood in uterine cavity.		*msl.cl.*	Circular muscles.
bl. r.	Red blood corpuscles.		*msl.lg.*	Longitudinal muscles.
bl. v.	Blood vessels.		*msl.rd.*	Radial muscles.
cap.	Capillaries.		*sh.*	Sheath.
dor.	Dorsal.		*str.*	Stroma.
ep.gl.	Glandular epithelium.		*v.*	Vein.
ep.ut.	Uterine epithelium.		*vent.*	Ventral.
gl.	Gland.			

The sections are cut through the body of the uterus at right angles to its antero-
posterior plane, and vertical to the wall of the uterus.

The figs. 1 to 11 are drawn with ZEISS' E. objective and No. 4 eye-piece; fig. 12
with ZEISS' a* objective and No. 2 eye-piece; figs. 13 to 30 and 32 to 39 with
REICHERT'S $\frac{1}{18}$th oil immersion objective and No. 4 eye-piece; and figs. 31 and 40
with the same objective and No. 2 eye-piece.

All the drawings were made with the aid of the camera lucida. In the coloured drawings the yellow colour represents blood; in most of the figures the blood corpuscles are not indicated individually.

PLATE 35.

Fig. 1. A section through the vertical wall of a uterus, Stage I. Showing a portion of the mucosa and a small portion of the inner muscular layer. The thin lines running from the deeper part of the stroma outwards are fibrils only present at this stage.

Fig. 2. A section through the ventral wall of a uterus, Stage II. Showing a portion of the mucosa and a small portion of the inner muscular layer. The nuclei of the superficial portion of the stroma are densely packed and fusiform in shape.

Fig. 3. A section through the dorsal wall of a uterus, Stage III. Showing a portion of the mucosa only. The dense layer of the stroma nuclei is more restricted, the blood vessels are larger, and many lie flattened out close beneath the epithelium.

Fig. 4. A section through the dorsal wall of a uterus, Stage IV. Showing a portion of the mucosa only. Many of the superficial vessels have broken down, and extravasated blood lies amidst the stroma network.

PLATE 36.

Fig. 5. A section through the dorsal wall of a uterus, Stage V. Showing a portion of the mucosa only. A lacuna in the midst of the stroma is seen, with extravasated blood around, in the network of the stroma. The nuclei of the stroma superficial to the dense layer, are again more rounded.

Fig. 6. A section through the dorsal wall of a uterus, Stage V. Showing a portion of the mucosa only. The vessels are more numerous, and larger and more congested. Much extravasated blood is in the stroma, and lacunæ are shown below the epithelium.

Fig. 7. A section through the lateral part of the ventral wall of a uterus, Stage VI. Showing a piece of the mucosa, and subjacent muscle layer. Large lacunæ are shown, which include nearly all the extravasated blood. Some free blood is seen in the cavity of the uterus, which has escaped through ruptures in the epithelial wall of the lacunæ.

PLATE 37.

Fig. 8. A section through the dorsal wall of a uterus, Stage VII. The lower part of the section shows a small part of one of the lateral walls of the uterus. A portion only of the mucosa is shown. Denudation is here represented,

and the severity of the process is shown. A few pieces of the uterine epithelium, $ep.ut_1$, remain *in situ*, the rest is cast off. The glands are much distorted ; the cavity of the uterus is filled with débris, which will form the menstrual clot. Extravasated blood still remains in the stroma, but most of it is now free in the uterine cavity. The vessels in the deeper mucosa remain intact, there is no extravasated blood in that region.

PLATE 38.

Fig. 9. A section through the dorsal wall of a uterus, Stage VIII. Showing a portion of the mucosa only. Recuperation of the epithelium is in progress ; it is much flattened, *ep.ut*. Pieces of the mucosa, *x*, are still being cast off ; but the denuding process is almost over. The menstrual clot was still in this uterus. Extravasated blood is present in the stroma tissue.

Fig. 10. A section through the dorsal wall of a uterus, Stage VIII. Showing a portion of the mucosa only. The epithelium is now re-formed, but is not yet cubical. The stroma layer is shrinking, but remains attached to the epithelium by long protoplasmic processes. Extravasated blood is still present in the stroma, but numerous capillaries are being formed.

Fig. 11. A section through the dorsal wall of a uterus, Stage VIII. Showing a portion of the mucosa layer and subjacent muscles. The epithelium has followed the stroma, and is now closely attached to the latter ; its cells are cubical and columnar, and it is folded in many places to form new glands. The vessels are more numerous, and larger than in fig. 1, but otherwise this uterus is very like a specimen of Stage I. There is now no extravasated blood present in the stroma.

PLATE 39.

Fig. 12. A section through the body of a uterus towards the close of Stage VIII. Showing the epithelium, glands, and stroma of the mucosa, the muscle layers and sheath, and the blood vessels in the external muscle layer. The vessels are not shown in the internal muscle layer, nor in the mucosa, for the sake of clearness.

Fig. 13. A piece of glandular epithelium from the same uterus drawn in fig. 1, Stage I. The basement membrane and ragged processes on the surface of the cells (cilia ?) are shown.

Fig. 14. A piece of uterine epithelium and stroma from the same uterus, Stage I. The delicate protoplasmic processes of the stroma are shown in connection with the epithelial cells. The nuclei are round or oval, and have a nuclear network.

Fig. 15. A piece of the densely packed stroma seen in fig. 2, Stage II. The elongated nuclei are shown, many of which are undergoing amitotic division, d. Three stages of division are shown by d_2.

Fig. 16. Cells from the same uterus, Stage II.

> a. Part of a capillary containing a leucocyte (*leu.*) and blood corpuscles.
>
> b. Part of a capillary showing the nuclei of the cells forming its wall, b_1, b_2, b_3.
>
> > b_1 is at right angles to the flat plane of the cell.
> >
> > b_2 is more oblique, and b_3 is a surface view of the nucleus of a cell forming the lower wall of the capillary, which the section cuts at the end of a curve.
>
> c. Series of elongated nuclei of the stroma, showing amitotic division. $c_{2, 3, 4, 5}$ different stages of division.
>
> d. Series of nuclei undergoing fragmentation. d_1 is a typical nucleus. $d_{2, 3, 4}$ show the fragmentation process.
>
> h. Series of small nuclei undergoing division.
>
> f_1. Is probably a similar cell to those of the c series, but shows fragmentation.
>
> The c series, h series, and f_1 are commonly seen ; $d_{2, 3, 4}$ rarely seen.

Fig. 17. Nuclei of the stroma, from the same uterus drawn in fig. 4, Stage IV. Showing amitotic division. They are commonly seen in the dense area of the stroma.

Fig. 18. Piece of glandular epithelium and stroma from deep down in the mucosa ; from the same uterus as fig. 17 is taken, Stage IV. There is a basement membrane but no sheath. The stroma nuclei are elongated in the region of the gland. They are not dividing.

Fig. 19. A piece of lining epithelium from the same uterus as fig. 18 is taken, Stage IV. The nuclear network is barely visible in some, and not at all in other nuclei. One large nucleolus is present at the base of each cell in place of the nuclear network and chromatin granules seen in fig. 14. The protoplasm of the cells is still continuous with the stroma network.

Fig. 20. A piece of the stroma from near the surface of the same uterus as fig. 7 is taken, Stage VI. Some nuclei, st_1, remain like those in fig. 14, but many, st_2, show degeneration changes ; they are shrivelled and stain darkly. The protoplasm of these latter cells is much reduced in amount, st_2.

Fig. 21. A piece of uterine epithelium from the surface of a lacuna, Stage VI., together with shrivelled stroma elements and blood corpuscles. The nuclei of the epithelium are also shrivelled and degenerating.

PLATE 40.

Fig. 22. An artery and vein from just below the dense area of the stroma, Stage III.
Showing the multiplication of the cells forming the walls thereof, a and v_1.

Fig. 23. Part of the wall of a capillary from near the surface of the mucosa, Stage IV.
Showing hypertrophy of the nuclei. This shows the condition of the cells
prior to rupture of the vessel.

Fig. 24. A ruptured capillary and stroma, from near the surface of the mucosa,
Stage IV. Hypertrophy of the nuclei and protoplasm of the stroma and
vessel wall is shown, and the wall of the vessel has broken down, allowing
the blood corpuscles to be extravasated in the meshes of the stroma. A
leucocyte remains attached to the remnant of the ruptured vessel. The
protoplasm of the cells is less sharply defined than it is in fig. 22.

Fig. 25. An artery and vein with stroma from the deeper part of the mucosa of the
same uterus as fig. 24, Stage IV. The vessels are not ruptured, but the
nuclei and protoplasm of the stroma and of the walls of the vessels
are hypertrophied, and strands of protoplasm are stretched across the
lumen of the vessels. A leucocyte is shown in the vein (*leu.*). The
other cell in the vein belongs to the wall of the vessel which has been cut
in section owing to the irregular bulging of the wall. The walls of the
vessels are not so solid and compact as they are in fig. 22.

Fig. 26. A piece of the superficial stroma at Stage VIII. Showing newly formed
capillaries. The nuclei and protoplasm of the cells are now no longer
hypertrophied.

Fig. 27, A and B. An artery and vein from the deeper part of the mucosa, Stage VIII.
Showing the recovery of hypertrophied vessels. The nuclei and proto-
plasm still show signs of the swelling seen in fig. 25, but not nearly to the
same extent.

Fig. 28. A piece of the upper part of the mucosa from the same uterus as fig. 11,
Stage VIII. Showing the newly-formed cubical epithelium; the nuclei
of the stroma are now almost the same as in fig. 14, but still somewhat
irregular in shape, and a definitely formed capillary is present.

Fig. 29. Stroma cells from the same uterus as fig. 9, Stage VIII. Showing, a, hyper-
trophied nucleus; $b.b.$, irregular nuclei, probably dividing; c_1, c_2, regular
nuclei dividing; d_1, d_2, fragmentation of the nucleus.

Fig. 30. Débris from the cavity of the same uterus as fig. 8, Stage VII. Uterine
and glandular epithelium, stroma nuclei, leucocytes, and red blood cor-
puscles; nearly all in degenerate condition.

PLATE 41.

Figs. 31 to 36 are taken from various uteri during Stage VIII., to illustrate the re-formation of the epithelium. The nuclei marked *d* are undergoing division.

Fig. 31. A section through a piece of growing epithelium connected with a gland at the lower end of the figure, *ep.ut.* The close connection between the stroma and the growing epithelium at the upper end of the section is shown, the two being in fact continuous there.

Fig. 32. A section through the growing point of epithelium, showing the amitotic division of the terminal cell.

Fig. 33. A section showing the flattened epithelium seen in fig. 9, and the scattered stroma nuclei with long protoplasmic processes, below. Blood corpuscles in great number are enclosed within the stroma network—extravasated blood. *x* is a stroma cell; *y*, a cell of the epithelial layer derived from the stroma; *z*, an epithelial cell.

Figs. 34 and 35. Sections of a slightly later stage, in which the flattened cells of the epithelium are becoming columnar. The nuclei are still of irregular shape and size.

Fig. 36. A section through the epithelium and superficial stroma, from the same uterus as fig. 10. Showing the more regular epithelium and the scattered nuclei and long processes of the underlying stroma. The nuclei of these layers are still irregular, but are more nearly approached to those seen in fig. 14 than heretofore. Minute capillaries are seen, *cap.*, and a few isolated blood corpuscles.

Figs. 37 to 40 are drawings of leucocytes.

Fig. 37 shows a leucocyte with stellate processes, adhering to the remains of an hypertrophied and broken down capillary, Stage IV.

Fig. 38. A colony of leucocytes within a vessel, Stage VI. The nuclei are in various stages of division. A sharp outline is invariably present round the cell; *d* indicates a cell whose single nucleus is dividing into two.

Figs. 39A and 39B are drawings of leucocytes which were situated within vessels at Stage VIII. Showing the various shaped nuclei seen, and leucocytes with ,one, two, three, and four nuclei within them; 39B shows a leucocyte whose single nucleus is dividing into four simultaneously—fragmentation. The chromatin within this nucleus is confined to the boundary-wall of the nucleus.

Fig. 40. Outline of a vessel, Stage VIII. Showing the very large proportion of leucocytes to red blood corpuscles, viz., 47·115 per cent. of leucocytes.

Fig 1

gl

bl v

l lg

ep ut

st

ep.gl.

Fig

bl v

art

art

art

ep gl

Fig 2

gl

ep ut

d.

l cl

msl lg.

Fig. 3.

gl.

Fig 7

Fig 6

Fig 5

ep ut

gl

bl v

leu

bl ex

lac

gl

bl fr

bl v

leu

msl lg.

msl cl

st

bl v

lac

Fig. 5.

l. v

ep ut

ep ut

st

ep gl

leu

st

•

Fig. 9

Fig 10

Fig 11

Fig 12

Fig 16

Fig 17.

Fig 18

Fig 19

Fig 20

Fig 21

Fig 23

Fig 24

Fig 26

Fig 28.

Fig 30

Fig 29

Fig 27a

Fig 27.

н

Fig 32

Fig 31

Fig 38
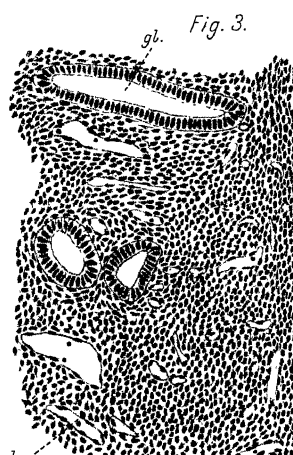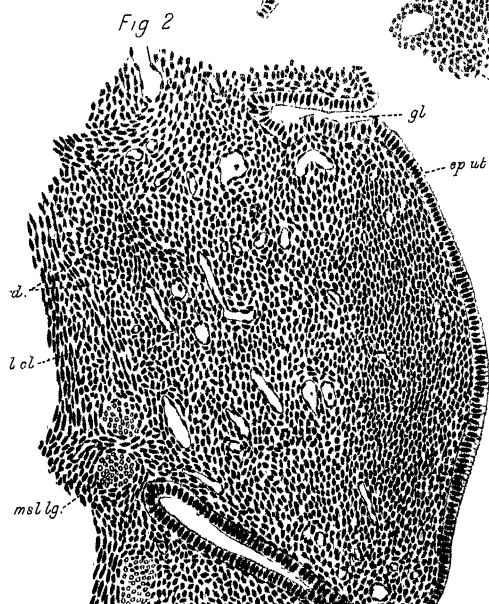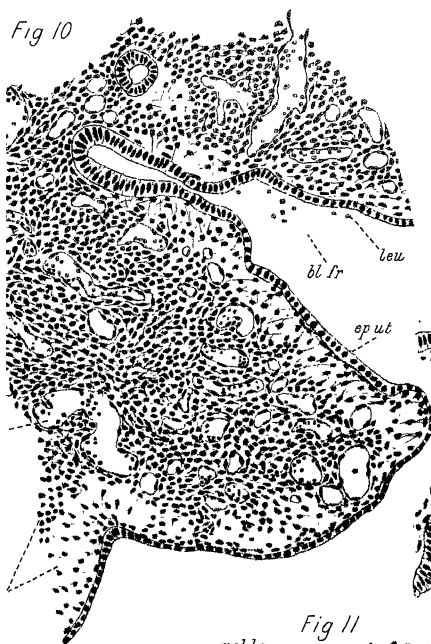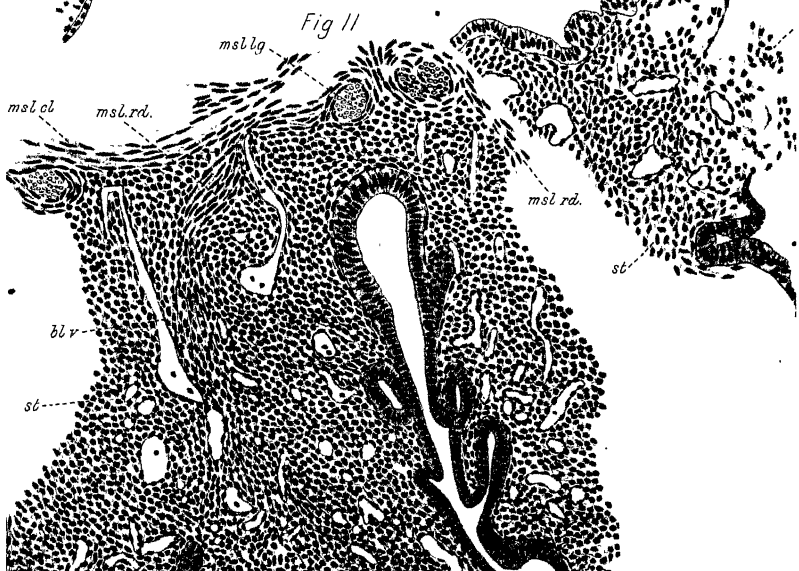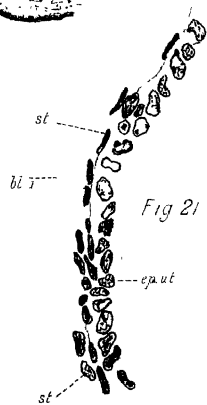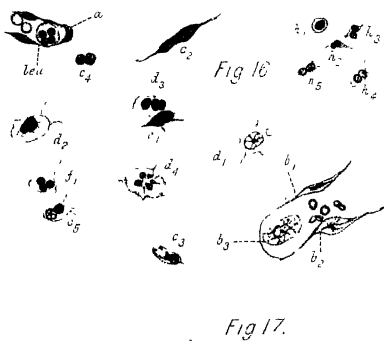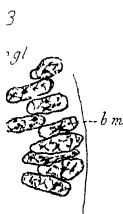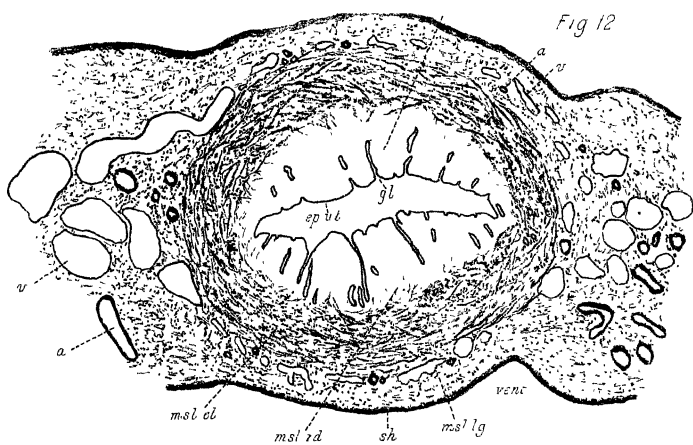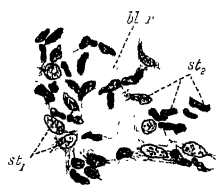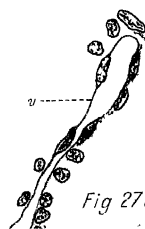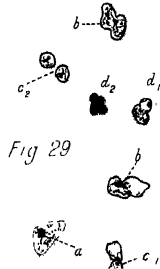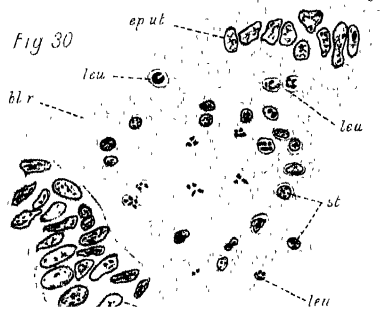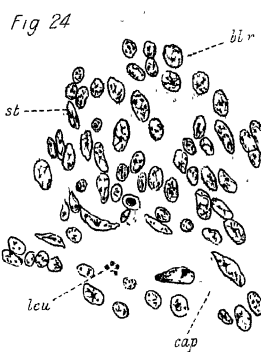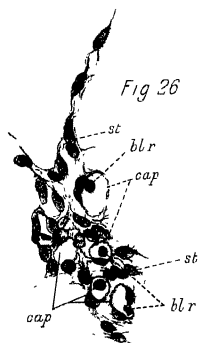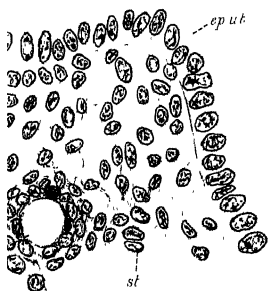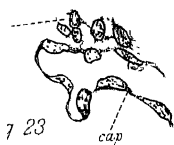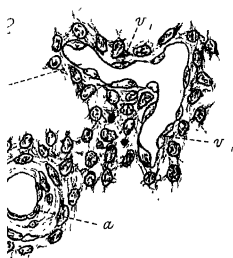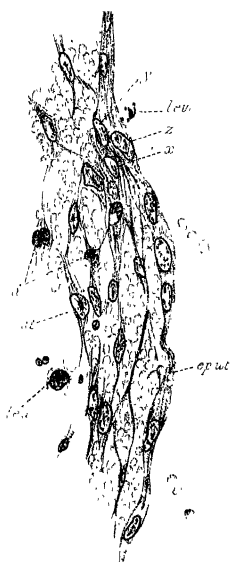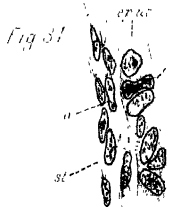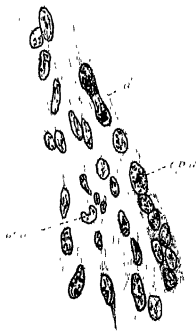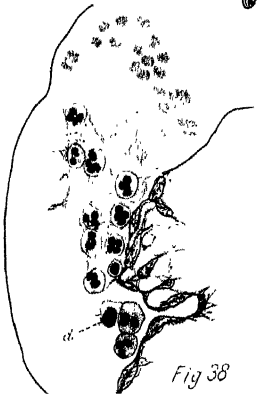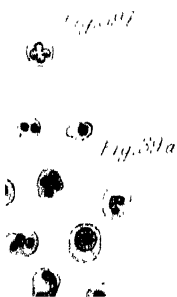
Fig 40

XII. *Studies in the Morphology of Spore-producing Members.—Equisetineæ and Lycopodineæ.*

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[PLATES 42–52.]

INTRODUCTION.

THE observations of HOFMEISTER stand, in their broad outlines, as the foundation of the morphology of archegoniate plants. It will be assumed that readers will accept in the main the homologies which, on the basis of those observations, he recognized between the corresponding parts of the Bryophyta, Vascular Cryptogams, and Gymnosperms; it will also be assumed that, whatever may have been the circumstances which led to it, antithetic alternation was brought about by elaboration of the zygote so as to form a new generation (the sporophyte) interpolated between successive gametophytes, and that the neutral generation is not in any sense the result of modification or metamorphosis of the sexual, but a new product having a distinct phylogenetic history of its own. Those who accept this view will keep distinct in their minds the sexual generation or gametophyte on the one hand, and the neutral generation or sporophyte on the other, whatever their variations, either in relative size or in physiological dependence; and they will recognize that no homologies are to be admitted between them or their parts. Clear conceptions on these points are absolutely essential, if there is to be real progress in comparative morphology; though the study of either generation may shed side-lights upon the problems relating to the other, the two alternating generations must be treated apart, so long as the main conclusions of HOFMEISTER continue to be accepted.

The assumption above made as to the origin of antithetic alternation is based upon a general comparison of living plants, which leads to the conclusion that, of the two alternating generations, the sexual generation (the oophyte or gametophyte) was the original one: that subsequently a neutral (the sporophyte) was produced as a stage gradually interpolated between the successive sexual generations; and that this new growth was produced, not by mere modification of the oophyte, or of a part of it, *but by amplification of the product of sexuality—the zygote;* by its sub-division into numerous

cells the effect of a single sexual process is distributed over the parts produced, these parts, when isolated, may be styled the *spores,* or, to distinguish them from other unicellular organs of propagation, they are designated by the special term "*carpospores.*" Further comparison of living forms leads to the conclusion that there followed upon the increasing size of the sporophyte a *progressive sterilization* of its tissues, so that only a part of those tissues continued to be sporogenous; this may be most readily illustrated by reference to the Bryophyta (see below, p. 485, &c.), a series of plants in which progressive sterilization of the tissues of the sporophyte appears to have been closely connected with its increasing size and structural complexity. The series is, however, characterized by the simple external form of the sporophyte, and by the further fact that the sporogenous tissue of each individual sporophyte is normally a continuous mass; accordingly, the recognition of the progressive sterilization is in them comparatively easy.

Progressive sterilization seems also to have played an important part in the evolution of the Vascular Cryptogams; in these, as we see them at the present day, the proportion of sporogenous tissue to vegetative tissue is comparatively small; the sporogenous cells are commonly separated into small masses, or are distributed singly; in fact, the sterilization appears to have progressed much further in the Vascular Cryptogams than in the Bryophyta; the external form, also, is much more complex, owing to the presence of appendicular organs. But though it may be more difficult to trace the steps of sterilization in the living Vascular Cryptogams, owing to the process having been more completely carried out, the experience gained from study of the Algæ and Bryophyta should direct the line of observation into this channel, and occasion to note its effects will frequently arise in subsequent pages.

The adoption of the foliar habit as seen in the sporophyte of Vascular Cryptogams, together with the elaboration of a subterranean system for absorption, brought with it very great advantages; the physiological independence of the sporophyte was thereby secured, and it accordingly assumed a more permanent character and longer life. Considering the greatness of the advantage thus gained, and the remote period of the past at which the step must have been taken in any one of the several series of vascular plants, it is intelligible enough that connecting links, showing how the appendicular organs were first acquired, and how the transition from a continuous archesporium to one consisting of isolated cells or cell-groups took place, should be few and uncertain among the plants of the present day. It has been remarked that the gap between the Bryophyta and the Vascular Cryptogams is the widest in the whole vegetable kingdom. On the one hand are plants with a dependent sporophyte of simple external form, with a continuous sporogenous mass, and having the sporogenous tissue in large proportion as compared with the sterile tissue; on the other, plants with the sporophyte physiologically independent, with complex external form, and sporogenous tissues in isolated masses, or appearing as isolated cells, and with the sterile tissue exceeding it greatly. To bridge over the gap between plants with

characters thus widely divergent is the most clearly outstanding problem of morphology. Hitherto, fossils have helped but little in this direction: it therefore appears that investigators must depend mainly upon comparison of living forms. It is not to be expected that even the most diligent study and comparison of these can do more than give suggestions as to the solution of the problem; but if the view above propounded be steadily maintained, and the more complex sporophyte be regarded as a derivative by partial sterilization, and vegetative amplification of simpler original forms, the gulf would appear a less serious one than it is at present commonly believed to be.

The most important additions to knowledge of the Vascular Cryptogams in recent years have related to the oophyte and sexual organs, and to the development of the embryo. While the importance of these is duly appreciated, I may remark that I have not complete confidence in phylogenetic conclusions based upon the vegetative characters of the gametophyte; that generation is certainly very plastic in its form, as witness the various types of prothallus in the genus *Lycopodium*, where the mature sporophyte remains much more constant in character. The question of the relative value of the characters of the two generations for purposes of comparison, in those plants which show conspicuous alternation will be discussed more fully towards the close of this memoir; in the meanwhile it may be stated that while due prominence will be accorded to the sexual generation, the characters of the neutral generation will here chiefly engage our attention, for it is believed that a widely extended study of the morphology of spore-production is specially needed at the present juncture; not only should a re-examination be made of sporangia already investigated by others, but also observations on such rare forms as have not hitherto been fully studied from the point of view of development.

The chief reasons for attaching special weight to the comparative and developmental study of spore-producing organs are as follows. Spore-production was undoubtedly the first office of the sporophyte, and to it practically the whole of the sporogonium was originally devoted; the sporogonium of the simpler Liverworts illustrates what was probably the primitive condition before the vegetative system of the sporophyte appeared. Spore-production is still, even in the most complex of the higher forms, the end to which the whole vegetative system tends. In the life-cycle of the vascular plants and Bryophyta of the present day the production of spores is a regularly recurring event,* and there is every reason to believe that it was regularly repeated throughout the course of their evolution. The spore-producing members, whatever their form, may therefore be styled *primary* as regards the history of descent, and the sporogenous cells which they contain are to be viewed as, at least,

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* Exception must be made in the case of those few plants which show the phenomenon of apospory, or in which propagation is conducted in a vegetative manner only; both of these conditions have probably been acquired at a comparatively late period.

the functional, and presumably also the morphological representatives, which survive from the original connected sporogenous mass.*

But the case is different with the vegetative organs of the sporophyte, such as axis, leaf, and root; on comparative grounds it may be concluded that these owed their origin to that progressive sterilization and amplification above alluded to; that a vegetative period, often of considerable length, has thus been intercalated between the fertilization of the ovum and the production of the spores, and thereby the date of production of the spores deferred. However great may be the size of the vegetative organs, the complexity of their form and internal structure, and however long the time of their duration, they should still be placed in a subordinate position, and may be styled *secondary* members as regards the history of descent. Priority of morphological importance for comparative purposes is habitually conceded to those parts which are of earliest date and of most constant occurrence; accordingly the priority must here be given, not to the vegetative parts of vascular plants, though these appear first in the development of the individual, but to the spore-producing members, the sporogenous cells of which are believed to represent that tissue which formed originally the whole constituent mass of the sporophyte.

Having thus stated broadly the point of view which will be maintained throughout this memoir, it will be well to look back upon earlier opinions as to the morphological "dignity" of the sporangium. The earlier expressions of opinion on the nature of sporangia related to the pollen-sacs and ovules of the higher plants, these being very readily observed. It has been the misfortune of the morphological branch of our science that it was hampered by the early possession of a fairly accurate knowledge of the higher forms and recognition of their parts; as the result, a definite terminology, as well as a certain attitude of mind regarding them, became fixed at a date prior to the investigation of the lower forms; every one now would admit that the morphology of the higher plants is to be read in the light of a knowledge of the lower types, and yet morphologists commonly retain such views as would never have gained acceptance, if the course of investigation had been inverted, and the history of the progress of the science had led from the examination of the lower forms to that of the higher. On the most fundamental ideas of the relation of the sporangium to the vegetative organs, the views commonly held are incompatible with the probable history of descent; such views are to be looked upon as a legacy left behind by those so placed in the history of the science that their progress in morphological study was downwards along the branches of the developmental tree, instead of upwards.

The first expression of opinion on the morphological nature of sporangia appears to have been that of WOLFF, and naturally relates to the higher plants. He states his

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* The question as to the sporogenous cells of modern sporangia being the lineal descendants, or, in other words, the morphological representatives of the sporogenous cells of a simple undifferentiated sporophyte will be discussed below, in the light of facts to be described later; at present, the view that in most cases they are so, may be taken as a working hypothesis strongly supported on general grounds.

view that "even the seeds, notwithstanding that at first sight they have not the slightest resemblance to leaves, are still in fact simply coalescent leaves,"* and again he concludes "that the stamens also are in their nature simply leaves. In one word, in the whole plant, whose parts at first sight appear so excessively diverse, we see, on mature consideration, nothing more than leaves and stem, since the root belongs to the latter."† From these quotations it appears that WOLFF, though thoroughly acquainted with the parts of the flower, did not distinguish sporangia as a separate category, but as modifications of leaves or parts of leaves.

The same was essentially GOETHE's view, arrived at by a distinct course of reasoning. GOETHE‡ (1790) at the outset drew attention to the fact that certain exterior parts of plants sometimes "change and pass into the form of adjacent parts either wholly or in greater or less degree." Of this change or "metamorphosis" he distinguishes three kinds, *regular, irregular, and occasional;* the third, since it includes only monstrous developments, may be at once dismissed. Under *regular or progressive metamorphosis* GOETHE included the changes involved in the development of the individual; the series, cotyledons, foliage leaves, bracts, and floral organs in an annual plant serve as an illustration. As *irregular or retrogressive metamorphosis* he designated such cases as the petaloid development of stamens, where parts which stand higher in the progressive series take, as they develop, the characters of a lower grade. In the recapitulation paragraphs, 115, 119, GOETHE sums up that, whether forming shoots, flowers, or fruits, it is still but the same organs which, under different names and various forms, answer the demands of nature; all are referable to one, viz., the leaf.

It is plain, from the expressions used by WOLFF and GOETHE, that the idea of the sporangium as an independent member was not yet suggested; the *pollen-sac* is regarded by GOETHE as the result of contraction of the margin of the staminal leaf (§47, p. 33); in writing of the ovules, he compares them with buds, and finally concludes (§93) that "the seeds, which differ from buds by being enclosed, and from gemmæ by the visible cause of their formation and separation, are still nearly related to both." Thus, notwithstanding that there was as yet no definite conception of the sporangium as an independent member, the theory of metamorphosis is intimately connected with the early views as to the nature of the sporangium ; it is to be remembered that the early writers on this subject, and especially GOETHE, had as yet no clear ideas as to descent, but had rather a bias towards a belief in the fixity of species; their conclusions are drawn from the study of the individual, not from a comparative study of plants at large; nor can we wonder that this should have been so at a time when the lower forms were hardly studied at all. As we

* WOLFF, "De Formatione Intestinorum," 'Novi Comment. Acad. Petropol,' Tom. 12, 1766–1767, p. 405.

† *Loc. cit.*, p. 406.

‡ GOETHE'S 'Werke.' SPEMANN, Berlin, vol. 33, p. 19, &c.

proceed it will be shown how this morphology, based on ontogeny, has left permanent and erroneous traces, which the modern views of phylogeny have not yet obliterated; and that in certain most essential points the two methods are at variance—in which case, views based on sound comparison must take precedence over conclusions drawn from the mere study of the individual.

During the fifty years which followed the publication of GOETHE'S essay, little was done to advance comparative morphology; the area of facts relating to the Phanerogams was extended, but in the light of our present knowledge it is plain that these could not be properly understood except upon a basis of comparison with the archegoniate plants. This is amply illustrated in the text-books of the period; in the concluding pages of the first volume of his 'Organographie Végétale,' AUG.-PYR. DE CANDOLLE (1827) re-asserts GOETHE'S conclusions, though with a wealth of fresh illustration; the sporangium is still an unrecognised part, and the members of the whole plant, including the parts of the flower, are referred to the three categories of Root, Stem, and Leaf. While earlier writers hardly attempt a comparison with Cryptogams, TREVIRANUS did so ('Physiologie der Gewächse' (1838), vol. 2, p. 461, &c.), but in the absence of knowledge of the life-cycle in such plants, the result was not satis-factory. A more successful comparison was however made by VON MOHL in a Dissertation published in 1837;[*] his main conclusion was that in all the Vascular Cryptogams examined, the structure of the sporangium shows an undeniable similarity to the theca of an anther. But as a basis for clearer views two things were necessary, a knowledge of the life-cycle of the higher Cryptogams, and of the development of the flower, and of its parts in the Phanerogams.

The latter of these subjects, though earlier investigated by WOLFF, was taken up with vigour by SCHLEIDEN, and his results were embodied in his 'Grundzüge der Wissenschaftlichen Botanik' (1845), a book which had a profound effect upon the progress of morphology. We there find (p. 282) the comparison between the stamens of Phanerogams, and the sporophylls of Vascular Cryptogams broadly stated; the pollen-sacs and sporangia, and the pollen-grains and spores are also regarded as homologous; the view is maintained that the stamen is of foliar nature, but instead of assuming a progressive metamorphosis, and stating, as previous writers had done, that the stamen is an altered *foliage* leaf, SCHLEIDEN, resting upon the sure basis of development, did not go further than to admit that the stamen is of modified foliar nature (p. 284, &c.). In the absence of a theory of descent, this is, doubtless, the furthest he could safely go; and it will be seen that he there approaches nearer to the views to be put forward in this paper, than most of the writers who followed him.

While thus drawing comparisons between the sporangia of the Vascular Crypto-gams and the pollen-sacs, and recognizing the stamen as of foliar nature, the writers previous to 1850 seem to have been content to place the ovule in a separate

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* 'Vermischte Schriften,' 1837, p. 94.

category; from GOETHE'S time onwards it had been regarded by most botanists as the equivalent of a bud, but this is not surprising when we consider the peculiar form and structure of the ovule, which differs so widely from typical sporangia. It was only through the study of the life-cycle of the Vascular Cryptogams and Gymnosperms, and comparison of the latter with the heterosporous forms of the former, that the true nature of the ovule could be approached. This work was accomplished by HOFMEISTER in his 'Vergleichende Untersuchungen,' 1851, and those results of his labours, which at present chiefly interest us, were the establishment of clear views on alternation of generations, and the recognition of the homology of the ovule of Phanerogams with the megasporangium of the heterosporous Pteridophyta. From this time the ovule had to be viewed as bearing the same relation to the pollen-sac as the megasporangium to the microsporangium. Shortly after the publication of HOFMEISTER's discoveries, the 'Origin of Species' appeared (1859), but it seems at first to have affected the discussions on morphology only to a slight degree. The acceptance of a theory of descent, together with an adequate knowledge of alternation, should have led quickly to the establishment of the sporangia as forming a distinct category of members; but this result was long deferred, and for fully twenty years the questions discussed related as before to the "morphological dignity" of the pollen-sac, and of the ovule; little difficulty was felt by certain authors in assigning the pollen-sacs to a different morphological category from the ovules; many even held that the ovule might have a different morphological character in different cases. SACHS ('Lehrbuch,' 4th ed., p. 482), after describing the various positions of the ovules, writes : " If we apply general morphological definitions to these relations we should in the first of the above cases have ovules of an axile nature, they would be mere metamorphosed caulomes; where they arise below the apex of the floral axis, they would be regarded as metamorphosed leaves; where they arise laterally out of the margins of the carpels they would be viewed as metamorphosed pinnæ; for those which spring from the surface of the carpels there is no clear analogy with purely vegetative structure, but we may here remember the sporangia of the Lycopodiaceæ; further, it even seems possible to regard many ovules, e.g., those of the Orchideæ, as metamorphosed trichomes, as in the case of the sporangia of Ferns and Rhizocarps." Some also held a similar view for the pollen-sac, asserting that while most stamens represent whole leaves, others are the result of metamorphosis of the axis itself (see EICHLER, 'Blüthendiagramme,' p. 47). It will be unnecessary here to follow out the controversies on these matters into their details; the position arrived at by those who admitted these divers morphological characters was, doubtless, due partly to the influence of GOETHE, and of his theory of metamorphosis, partly to the effect of SCHLEIDEN's insistence on the importance of the study of development of the individual in the solution of questions in morphology. Thus, the sporangium, whether it were that of a Vascular Cryptogam, or the pollen-sac or the ovule of a Phanerogam, was still considered as being borne by, or the morphological equivalent

of, some vegetative organ, according as it was found to be comparable to one or another of these, as regards its position, and mode of origin.*

This method of morphology takes into account the individual development; it rests on observations of ontogeny, but leaves out of account the opinions as to descent. It was not likely that such a method should long stand unassailed after evolution had come to be accepted. Though ALEX. BRAUN had already ascribed to the ovule the uniform character of a bud, it was STRASBURGER who appears first to have approached the question of variable morphological value of stamens and ovules from a general point of view.† He lays down at once the principle that "it is the highest problem of morphology to explain the form of plants, but this problem can only be solved genealogically." Having thus made descent the foundation for his morphology, he pointed to the general uniformity of construction of ovules, and expressed the opinion that from the phylogenetic aspect it is not to be imagined that such organs could have been formed at different times, and in different ways. He held that they were formed once only, among Phanerogamic plants, and have but one morphological character, viz., that of a bud, of which the nucellus is the axis and the integuments leaves. This opinion was shared by EICHLER,‡ who also on the grounds of descent, believed that such a structure as the ovule must have throughout the same morphological character; he too regarded it as a bud, assenting thereby to the position originally taken up by GOETHE. It was, however, a decided advance to have introduced the idea of descent into the argument, and to have dismissed the views of variable morphological character of the sporangia; but STRASBURGER did not follow his views out to their logical conclusion; the ovule, which is a megasporangium, is recognized by him as a bud; it corresponds to the megasporangium of heterosporous Vascular Cryptogams; are they also metamorphosed buds? The megasporangium and microsporangium of such forms are believed to have originated by differentiation of a uniform type of sporangium, are then all sporangia buds? Even the pollen-sac is a sporangium; is then a pollen-sac a bud? In order to be consistent this should be the interpretation of it by those believers in descent who also hold that the ovule is a bud. Evidently STRASBURGER when writing his great work on the Coniferæ and Gnetaceæ was still imbued with the old idea of metamorphosis; he still spoke of the three fundamental structures (p. 441), and obviously looked upon the sporangia of the higher plants as the result of metamorphosis of

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* SACHS, in his 'Lehrbuch,' 4th ed., which may be taken as fairly representing the spirit of the time of its publication (1874), makes the general statement (p. 153) that "every organ is either a stem (axis), or leaf, or root, or hair." But it is especially remarked later (p. 155) by way of limitation, that "the statement," e.g., that "the sporangia of Ferns are trichomes, only means that they arise, like all hairs, from epidermal cells; hairs and Fern sporangia are in this respect morphologically equivalent." Morphological equivalence is, in this sense, simply equivalence of development in the individual; origin, by descent, is not taken into account in determining it.

† 'Coniferen und Gnetaceen,' p. 396, &c.

‡ 'Blüthendiagramme,' p. 45.

parts typically vegetative. It remained for GOEBEL to take the next step towards placing the morphology of the sporangium on a sound basis : rejecting the view which had been held all along, from the times of WOLFF and GOETHE, that in one form or another, the sporangium is the result of metamorphosis of some part of the vegetative system, he plainly stated that the sporangium is not referable in its origin to any other category of members. "As a shoot remains a shoot, and does not lose its morphological value (dignity) whether it arise as a lateral shoot on the growing point of a stem, or from the embryonic tissue of a foliage leaf (as in many Ferns), or is adventitious on a root, &c., so also a sporangium remains a sporangium and nothing else, whatever its position ; sporangia are just as much organs *sui generis* as are shoots, roots, &c."*

The above conclusion opened a new page in the history of the morphology of the sporophyte ; a position was thus attained which, for the first time, became intelligible from the point of view of descent. Spore-production having been, as we must believe, a constantly recurring event during the whole course of evolution, and having preceded the vegetative development of the sporophyte, it was not possible to understand how the organs in which spore-production took place, could be merely the result of metamorphosis of those vegetative parts which, on grounds of phylogeny, there is every reason to believe were of later origin. By rejecting the old opinions, and recognizing the sporangium as an independent member, the way was at last cleared for a morphology based upon, or at least in harmony with, the conclusions as to descent, not upon the mere study of the development of the individual.

The question as to the relative importance of observations of the ontogeny, and conclusions as to phylogeny, is one of the most critical in all morphology, for on it depends the whole basis of the system : provided the conclusions as to phylogeny be sound, they should, in my opinion, have the precedence. But in practice it has been customary to hold the reverse, and to take the development of the individual as the basis of morphological treatment, while such views on descent as are based on comparison are often left practically out of account, or given only a secondary place. It will be useful to dwell upon this point so that readers may enter upon the subsequent discussion with an open mind.

From the time of GOETHE, observations of the development of the individual have had a strong influence upon morphological speculation. His introduction of the terms progressive and retrogressive metamorphosis, though they had at the time no clear meaning as to descent, doubtless gave a bias in a certain direction. SCHLEIDEN's insistence on the importance of developmental data turned the current of observation into that direction. When evolution came to be recognized, and the embryology of animals was found to justify for them the acceptance of the recapitulation theory, GOETHE's views were read in a new light, and the successive phases of the individual life of the plant also naturally appeared to illustrate, in a measure, the history of its descent; accordingly,

* 'Bot. Zeit.,' 1881, p. 701.

speaking at present only of the sporophyte of the higher plants, the cotyledons might be viewed as the earliest type of leaf, the foliage leaves the next, and finally, the floral leaves and sporophylls. Any case of mere vegetative development of a sporophyll would be described, almost in the words of GOETHE, as a sample of retrogressive metamorphosis, or in the language of descent, as a reversion.

A different position was taken up by the adherents of what has been termed the "differentiation theory";* for them the various forms of leaf are the result of differentiation of an original type : it was found that in their earliest developmental stages all leaves are virtually alike ; here, it was maintained, might be recognized the fundamental form which underlies all the various lateral organs, while the development of these organs themselves was accepted as in fact a real metamorphosis, a differentiation to subserve various functions. The actual metamorphosis of one specialized organ into another was however denied ; thus to the adherents of this theory stamens are by no means metamorphosed foliage leaves. GOEBEL, however, rightly points out "that such a metamorphosis of one form of leaf to another may occur," and cites his own experiments, by which leaves, normally developing into scale-leaves, were transformed in the course of their individual growth into foliage leaves.† He further cites those interesting cases of the appearance of sporangia on leaves of *Botrychium*, which are normally green expanded foliage leaves, noting that, where the sporangia occur, the vegetative development is reduced : similar observations on *Osmunda* and *Blechnum* are also quoted, and from these the conclusion was drawn that "the sporophylls are metamorphosed foliage leaves"; and still more explicitly it is stated for plants at large (*loc. cit.*, p. 118), "that the plant forms in the first instance leaves of only one type, the foliage leaves, though their growth is often modified by influences which appear in the course of development." But the facts do not necessarily bear out the interpretation thus put upon them : what is shown by such cases as that of *Botrychium*, or *Blechnum*, or *Osmunda*, is simply that a correlation exists between spore-production and vegetative development : where sporangia are present the vegetative growth is arrested, where sporangia are absent vegetative growth is more active : the further step that the sporophyll is a metamorphosed foliage leaf, and that the plant in the first instance forms only foliage leaves is an assumption, made in accordance with the custom of reading conclusions of phylogeny into the history of the individual, but without sufficient regard for wide-extended comparison. I do not admit that the priority of the foliage leaf in the development of the individual shows its priority in the history of descent : these observations of correlation do not appear to me to touch the question of priority of evolution.‡ I shall attempt to show on a basis of comparison of certain of the lower Vascular Cryptogams that, *in some cases at least*, foliage leaves may probably have

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* HANSTEIN, 'Beitr. z. allg. Morph. d. Pflanzen,' 1882, &c.

† 'Bot. Zeit.,' 1880, p. 753, &c., also SCHENK's 'Handbuch,' vol. 3, p. 110.

‡ On this subject, compare 'Annals of Botany,' vol. 7, p. 373.

been the result of sterilization of sporophylls, and I have little doubt that this mode of origin of foliage leaves has been a wide-spread one. I should be slow, however, to make a general statement that either the one or the other was the original form of leaf for all vascular plants, for the different lines of descent have probably been too divergent to allow of a simple statement covering all cases : and it seems possible that at least the primary leaves of the protocorm* may have originated in a different way from other foliage leaves.

The idea of metamorphosis thus entertained by GOEBEL is primarily an *ontogenetic* one, but " it is extended by acceptance of the theory of descent."† In my view it has been customary to attach too great importance to observations of individual development, while too little attention has been devoted to broad comparison of the lower forms of the sporophyte, and to the phylogenetic conclusions which may be based upon such comparison ; these should serve as a guide, as well as a check, upon the interpretation of the individual development. If the development of the individual be constituted the chief basis of the morphology of the sporophyte, while the check of more general comparison be only slightly applied, the conclusion may be arrived at that the first parts to appear were vegetative organs of simple type, then more complex vegetative organs, and that the spore-bearing members were of subsequent origin ; it might also be concluded, with GOEBEL, that the sporophyll is a metamorphosed foliage leaf, or, in general terms, that spore-bearing members are the result of metamorphosis of pre-existent vegetative parts. But such a conclusion is plainly inconsistent with the story of the origin of the sporophyte, as it may be gathered from comparison of the lower archegoniate forms ; for in them the production of spores is present *from the first,* and appears even to have preceded the simplest vegetative development. In such a case as this, where conclusions from the study of development are in antagonism to those derived from comparison, the former should, in my opinion, give way to the latter.

Moreover, the custom of reading the development of the individual as illustrating the phylogenetic history, is, at best, based only on experience of its truth in certain cases ; because the epitome is found to be often true, it cannot be argued that it will be always true. In the rise of the sporophyte generation in archegoniate plants, I think we should hold, on grounds of broad comparison, that the development of the individual sporophyte cannot be taken *en bloc* as illustrating the history of the origin of the sporophyte at large. While spore-production appears to have been a constantly recurring fact from the first, comparison leads to the conclusion that the extensive vegetative phase which often precedes spore-production in the individual, was of more recent origin, appearing as an intercalated phase in the life-history. Accordingly it seems to me improbable that a sporophyll is really to be regarded phylogenetically as a metamorphosed foliage leaf; the converse would appear more reasonable.

* Compare Treub, ' Buitenzorg, Annales,' vol. 8, p. 1 &c.
† *Loc. cit.*, p. 113.

The attempt must be made to formulate in the mind some idea of how the more complex forms of sporophyte came into being; such an attempt it is now proposed to make, and to arrive, primarily on the basis of general comparison, and in the second place on the ground of individual development, at some idea how the progression from the simpler to the more complex forms of sporophyte may have taken place.

In briefly stating the main problem certain views will, as already intimated, be assumed to be generally accepted; they are as follows :—

1. The homology of the sporophyte as a whole in such plants as show antithetic alternation.

2. The absence of any homology between the sporophyte and the gametophyte, or their parts.

3. The derivation of the sporophyte of vascular plants from some simpler form, more or less like that of the lower Bryophyta.

4. The recurrence of spore-production as a constant event in each life-cycle, during evolution of archegoniate forms.

5. That, other things being equal, an increase in the number of spores produced is an advantage.

It is believed that all the above theses would be upheld by botanists at large.

The main problem is to frame a reasonable theory of the mode of derivation of the simpler vascular plants, with foliar appendages, and discrete archesporia, from forms with no appendicular organs, and an undivided or concrete archesporium.

There are at least three imaginable ways in which the numerous isolated spore-producing parts characteristic of vascular plants may have originated from simpler ancestors with concrete archesporium :—

(1.) By subdivision or partitioning of an original concrete archesporium, and outgrowth of the isolated fragments, together with the tissue surrounding them; sterilization of a part of the sporogenous tissue might play an important part in bringing this about.*

(2.) By branching of a sporogonium (chorisis) and duplication of the head, as is seen in some monstrous Moss-sporogonia.

(3.) By a formation of entirely new archesporia at points where such were not present in forms believed to represent their ancestors; such archesporia would have no direct connection by descent with pre-existent ones, but might in our view of sterilization be recognized as reversions.

It has been one object of the enquiry detailed below to see if among the lower vascular plants evidence can be found of any, or of all of these processes.

* It should here be remarked that the sub-division thus suggested may be further complicated by the continued apical or intercalary growth of the part concerned; and, as in the development of shoots and leaves, the increase in number of appendicular parts may be closely connected with such continued growth, so, I conceive, may also the number of spore-producing members. An application of this will be found at p. 534, &c.—F.O.B., February, 1894.

The great gulf between the Bryophyta and the Vascular Cryptogams must be faced sooner or later. It seems to me useless to turn away and say that the gulf is not to be bridged by hypotheses, and that the tracing of homologies in the sporophyte is hopeless, while our knowledge of the details of structure and development of those very forms which we believe to be near the boundary line is still so far from being complete. It is true that direct connecting links are almost absent; that fossils afford little help, and cannot be expected to yield much in the way of developmental data. · But the world's surface has been almost completely over-run, without disclosing in recent years new forms which would obviously take an intermediate place, so that it would be idle to wait indefinitely for the discovery of new forms to help us in the solution of the difficulty. It, therefore, appears to me to be a duty to turn to the living vascular plants nearest to the border line, and endeavour, by careful comparative study of them, to add to the knowledge of their details, with a view to attaining a basis for a reasonable hypothesis.

In pursuing this investigation, it will be important to fix the attention on organisms lower in the scale rather than on the higher forms; for it is essential not to obscure the issues by irrelevant references to the parts of organisms higher in the scale, such parts being often described by names which bear preconceived ideas with them; accordingly, notwithstanding the attractions which such comparisons present, allusions to the Phanerogams will for the present be almost entirely omitted, and the application of the results obtained to the explanation of the forms of the higher plants will be deferred to a future occasion. The first step will accordingly be to briefly review such facts relating to the Algæ and Bryophyta as, being already in our possession, may have their bearing upon the main points of our enquiry.

STERILIZATION IN THE ALGÆ AND BRYOPHYTA.

Reference having been made above to the part played by sterilization in the development of the sporophyte in the Bryophyta, and its progressive steps being more obvious in those plants than elsewhere, it will be well to examine the facts already recognized in them, before proceeding to the study of the Vascular Cryptogams. There is good reason to believe that the latter were derived from some Algal-Bryophytic ancestry, so that observations of progressive elaboration in the Algæ and Bryophyta may be reasonably accepted as suggestive of similar progress in the course of evolution of vascular plants.

In the simplest plants showing antithetic alternation, viz., certain Confervoideæ, the zygote undergoes on germination a subdivision of its protoplasmic body into a number of parts; there is, however, no differentiation of these, all are alike capable of acting as carpospores, and producing new individuals. Differences may be recognized between different types as regards the presence or absence of cell-walls; thus, in *Oedogonium* or *Sphæroplea*, the carpospores may be primordial cells, while in

Coleochæte they have a definite cell-membrane ; this is, however, promptly left behind by the escaping protoplasmic body. In these Algæ, the existence of the sporophyte is barely sketched out, and the parts of it show no differentiation ; it is even an open question whether this rudimentary sporophyte of certain Algæ be truly comparable with that of the Bryophyta ; but in any case it occupies the same position in the life-cycle.

Differentiation is, however, present even in the simplest Bryophytic forms, as ·in *Riccia glauca,* where the peripheral layer of cells constitutes a temporary wall round the undifferentiated mass of sporogenous cells within (comp. KIENITZ-GERLOFF, ' Bot. Zeit.,' 1874, Plate 3, figs. 1–6). Further steps in advancing complexity are illustrated in other Liverworts ; these may involve (*a*) sterilization of the whole transverse thickness of tissue of the sporogonium, proceeding from the base upwards, or (*b*) in differentiation of cells of the potential sporogenous tissue which remains, and sterilization of certain of them. In the rare genus, *Oxymitra,* the sporogonium is virtually similar to that of *Riccia,* but the cells of the wall appear to be less regular and definite, while certain of the cells of the sporogenous inner mass remain sterile,˙ but without undergoing any special development (LEITGEB, 'Lebermoose,' Heft 4, pp. 42–45, &c.). In *Corsinia,* also, such sterile cells are found, while here the complete sterilization of the lower part of the sporogonium shows a clear distinction of apex and base, which was absent in the earlier-named genera. In *Boschia,* the sterile cells among the spores take the elongated form, and spiral or annular thickening, appearing, when mature, as the well-known elaters. Thus, within the Riccieæ, both types of sterilization, (*a*) and (*b*), are initiated ;[*] in the Marchantiaceæ these characters become more marked, the elaters especially being a prominent feature, and showing some regularity of arrangement.

As regards (*a*), the sterilization of the whole thickness of tissue of the sporogonium, this is found in *Marchantia* to involve the whole hypobasal half of the embryo, the result being a pad of sterile tissue of the foot ; but in Jungermannieæ the hypobasal half remains small, while the epibasal half dividing into octants, each of these undergoes repeated transverse division and great elongation ; all the lower part of the tissue thus produced is vegetative, while the spore-production is confined to one or two tiers of cells at the apex. Here, then, is extensive sterilization extending far upwards into the body of the sporogonium—an intercalation of an intermediate vegetative stage between the zygote and spore-production. In the remaining family, viz., the Anthoceroteæ, the case as regards the type (*a*) of sterilization appears very similar to that in the Marchantiaceæ, only the hypobasal portion of the embryo being completely sterile throughout the whole transverse section, and forming the enlarged haustorial foot.

˙ Turning to the type of sterilization (*b*), viz., differentiation of the potential sporo-

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[*] It may be a question whether the genera of Algæ and Liverworts quoted really illustrate their phylogenetic origin. .   ˙

genous tissue and sterilization of certain of the differentiated parts, we see considerable differences in the disposition of the sterile parts. In the simplest cases the sterile cells do not differ from early arrested spore-mother-cells (*Oxymitra*); or they may be elongated and specially thickened, as elaters; these may be arranged with no regularity (*Boschia*), or they may radiate from the basal limit of the capsule (*Marchantia*); or project almost horizontally inwards from the wall of the spherical head (*Jungermannia trichophylla*, HOFMEISTER, 'Higher Cryptog.,' Plate 9, fig. 33); or they may appear as vertical rods or trabeculæ passing from the outer wall to the base of the cavity (*Frullania dilatata*, HOFMEISTER, *loc. cit.*, Plate 12, fig. 9, or *Lejeunia serpyllifolia*, LEITGEB, *loc. cit.*, Heft 1, Plate 1, fig. 14).

In the examples hitherto cited there is no association of the sterile elaters in masses; this is seen to occur, however, in various genera, and with interesting differences of detail. HOFMEISTER ('Higher Cryptog.,' p. 38), in describing the head of the sporogonium of *Pellia epiphylla*, remarks that "a whole string of cells lying in the longitudinal axis of the young fruit assumes this spindle form; around this string the rest of the cells destined to form elaters are arranged, radiating upwards. LEITGEB ('Lebermoose,' Heft 3, p. 57) points out that this axile strand does not extend further than about two-thirds of the distance from the base upwards through the cavity of the capsule; he also recognizes that the strand consists of grouped elaters. In certain other genera (*Metzgeria* and *Aneura*) a somewhat similar arrangement is found, but in an inverted position. LEITGEB states (*loc. cit.*, p. 31) that in "*Metzgeria* a group of cells at the apex, situated in contact with the wall of the capsule, is arrested in its growth without being transformed either into elaters or spore-mother-cells"; and, again, on p. 40, "the rows of spore-mother-cells and elaters do not converge to a point at the apex, but to a group of cells below it, composed of cubical cells, and there is no doubt that these correspond to the original internal cells of the upper storey of the spore-producing space." In *Aneura** also a somewhat similar central sterile mass is found attached to the wall at the apex of the sporogonium, and extending for a distance down into the spore-cavity as a solid column, but finally branching off into numerous separate elaters. In *Aneura pinguis* the relatively large sporogonium has a well-defined columella of sufficient size to be easily seen with the naked eye, and extending two-thirds down the cavity of the sporogonium: in *Aneura multifida* the sporogonium is smaller, and the columella is represented only as a minute mass of cells, projecting downwards about one-eighth of the distance through the cavity: from these incomplete columella-like masses the elaters radiate downwards into the mass of spores. The transition from the parenchymatous cells of the columella to typical, spindle-shaped, spirally-thickened elaters is beautifully illustrated in *A. multifida*, and may also be traced, though not with the same completeness, in *A. pinguis*. In these sporogonia also it would appear that the columella is the result

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* I am indebted to Professor FARMER for sections illustrating these points in *Aneura*, and for drawing my attention to them.

of massing together of sterile elaters, and it is to be noted not only that the columella is larger and more complete in the species having the larger sporogonium (*A. pinguis*), but also that the columella is more elaborate here than in the examples previously quoted.*

These very interesting cases of apparent grouping of the elaters to form a solid sterile axile strand, extending part way through the cavity, or even a sterile mass of ordinary cells, not developed as elaters, depending from the apex, appear to me to illustrate a matter of some importance. I think we may recognize in them imperfect steps towards the formation of a complete columella in the centre of the capsule, and see therein illustrated how, by the collection of sterile cells, isolated and uniformly diffused in other types, into a central position, a solid column of such tissue may be the result; such columns are here incomplete, but they suffice to suggest a possible mode of advance in sterilization, and it will be seen that this idea had not escaped the mind of LEITGEB.

In the Anthocoroteæ the sporogonium attains a greater complexity than in other Hepaticæ, but it is not uniform in the different genera, and the variations in structure have made them the subject of a careful discussion by LEITGEB ('Lebermoose,' Heft 5), from which the following facts and quotations are extracted.

In the more complex genera, *Anthoceros* and *Dendroceros*, the lower part of the sporogonium is developed as a foot, which keeps up physiological connection with the gametophyte; there is no seta, but the whole of the upper part of the sporogonium is composed of a columella, round which, and arched like a dome over its extreme apex, is the sporogenous layer, while the sporogonial wall, of several layers, covers this externally. The central cells, formed by periclinal division from each transverse tier of four in the young embryo, are entirely converted into the columella, the sporogenous layer being cut off from the outer cells by their first periclinal division. But of the sporogenous layer itself the majority of cells are sterile, while only the minority produce spores; the sterile cells in many species form a connected network, in the meshes of which the spore-mother-cells lie (*loc. cit.*, p. 6). The intercalary growth at the base of the sporogonium is apparently unlimited.

In the genus *Notothylas* not only are the characters different from the above, but there is also a most interesting variation in structure within the genus itself. The intercalary growth appears to be limited, when the limit occurs early, dwarf capsules, which are found in all species, are the result; sometimes, however, the growth is long continued. In certain species some of the capsules are like those of *Anthoceros* as regards the columella; but "in all species of *Notothylas* capsules are found, in which a columella is present it is true, but the cells of the columella are quite similarly

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formed to the other sterile cells of the capsule-cavity, and are very easily separable. Investigation of such capsules shows that the columella is not formed independently as in *Anthoceros,* and " separately from the spore-bearing layers, but arises by secondary differentiation within the spore-bearing space, and thus coincides in this respect with the columella of the Moss-capsule " (LEITGEB, 'Lebermoose,' vol. 5, p. 7). " But in some species of *Notothylas* (perhaps in all ?), there are also capsules in which the columella is absent from the first ; the sterile cells together form a connected tissue, a network which uniformly fills the whole cavity of the capsule . . . It is instructive that such capsules differ only by gradual steps from these with a columella which have just been described." . . . " By the formation of such columella-less capsules *Notothylas* is directly connected with other Liverworts." (LEITGEB, *loc. cit.,* vol. 5, p. 8.)

" If now, as I have endeavoured to show as probable, *Notothylas* be regarded as a form related to the Jungermanniaceæ, this must clearly be the case also for all the Anthoceroteæ, which are directly allied with *Notothylas,* and are doubtless descended from a similar form. The sterile cells at first uniformly distributed through the whole spore-cavity, though connected together, first united at the axis of the capsule to form a strand of cells the elements of which were not as yet different from the rest of the sterile cells. As above noted, such capsules are still found in *Notothylas.* Probably the next step was that the mode of development of the cells composing the axile strand differed from that of the cells outside, in the spore-cavity. Probably this form also is present in *Notothylas,* and I refer to the capsules described above which show a development of the columella similar to that of *Anthoceros.*" (LEITGEB, *loc. cit.,* p. 10.)

In the passages thus quoted, LEITGEB's view of the progression of sterilization in the Anthoceroteæ is quite plainly stated ; he distinctly recognizes the association of sterile cells together to form sterile masses as exemplified by the columella ; similar though less complete examples have been above cited in *Pellia, Aneura,* and *Metzgeria,* and we shall conclude that such association has been a feature in the advance of structure of the sporogonia in various genera of Hepaticæ.

There is one further point as regards the Anthoceroteæ which will be of theoretical interest : of the products of the sporogenous layer the majority of cells are sterile. Now it is quite possible and conceivable, after what has been noted above, that these sterile cells might further be associated together to form septa, which would thus partition off the sporogenous cells into separate compartments. We have no example of this among Bryophyta, but it would be the next natural step in continuation of the sterilizing process above traced.

The Mosses present fewer points of interest in this connection ; a certain parallelism may be traced with what has been described above—a progression of complexity from the simpler types of Phascaceæ to the Bryineæ ; and again it may be noted that whereas the archesporium in *Sphagnum* is of a closed dome-like form,

that of the Bryineæ is like a barrel open at the ends, and it may be suggested that this latter condition is the result of sterilization of the apical part. But in the Mosses the whole sporogenous layer bears spores; sterile cells appear to be absent from it, and if the potentialities of further development of those sterile cells be such as have been suggested in the previous paragraphs, then such potentialities will not have a place among the present living Mosses. From a theoretical point of view, however, the differences in place of origin of the archesporium are important; in *Sphagnum* it is derived from the amphithecium, in most Mosses it originates from the endothecium, and a similar difference of detail being also seen in the *Anthoceroteæ*, we have good reason to conclude that the actual layer which gives rise to the archesporium is not to be strictly defined for all Bryophyta, we shall thus be prepared to think it possible that the spore-production might be relegated to still more superficial positions in other plants, as is actually the case in the Vascular Cryptogams.

The elaboration of form of the capsules of some Mosses is interesting, as showing organisms making the best of a type of construction which is essentially unfitted for carrying on assimilation on a considerable scale. The whole sporogonium of *Anthoceros* is green, and can assimilate, though in most Liverworts assimilation is carried on only in a minor degree in the sporogonium, or not at all; and even in *Anthoceros* there is no specialization of form, and little specialization of structure to this end. In certain Mosses, however, there is more specialized structure: the seta usually contains little chlorophyll, but the head of the sporogonium, and especially the outgrowth of the apophysis where present, is the part in which assimilation is most active. The development of the apophysis for this purpose reaches its highest type in *Splachnum luteum* (compare VAIZEY, 'Annals of Botany,' 5, p. 1), in which it appears as an expanded disc, bearing numerous stomata, and filled internally with spongy chlorophyll-containing parenchyma. This is the most elaborate type of construction for assimilating purposes seen in the sporophyte of Bryophyta, and it is clearly the result of a comparatively slight modification of parts, already sterile, of the ordinary type of sporogonium.

In the above paragraphs some of the main phenomena of advance in the sporogonium of the Bryophyta have been briefly described, and it seems probable that progressive sterilization of sporogonous cells and progress of complexity of the sporogonium have gone hand in hand. As we recognize that vascular plants probably originated from some Algal-Bryophytic ancestry, it will be useful to carry forward to the study of the former clear ideas of the modifications which appear to have played a part in the advance of the Bryophyta. It is possible that there may be no homologies, possibly only slight analogies, but we should at least prepare ourselves to appreciate them if they exist.

The comparative study of the sporophyte of the Bryophyta leads to conclusions which may be formulated as follows:—

I. There is evidence of the sterilization of potential sporogenous cells, and the first result was probably the formation of a protective peripheral wall (*Riccia*).

II. In the space within this wall other sterile cells may be dispersed among the sporogenous cells (elaters, &c.).

III. Sterile cells similar to those thus isolated may in some forms be grouped together, producing coherent sterile masses of tissue (columella of *Pellia*, *Metzgeria*, *Aneura*, and Anthoceroteæ).

IV. In all the more complex forms a central sterile columella exists, while the spores originate from a definite band outside it (the archesporium).

V. The position of this band is not constant, originating sometimes more deeply, at other times being nearer the surface.

VI. The archesporium may give rise to both sterile and fertile cells (*Anthoceros*).

VII. In the lower part of the sporogonium a complete sterilization of the whole thickness of the sporogonium may be seen. This involves, first, the hypobasal half of the embryo, as in Marchantiaceæ; or, progressing to successively higher tiers of cells in the epibasal half, forms the sterile seta of the Jungermannieæ.

VIII. Intercalary growth may play a prominent part both in the sterile (*Junger-mannia*) and in the fertile parts (*Anthoceros*).

IX. Elaboration of external form is suggested (*Polytrichum*, *Splachnum*), but never carried far.

X. Assimilation may be actively pursued in the sterile tissues, either in those of the seta (including the apophysis, *Splachnum*, &c.), or in the fertile part of the sporo-gonium (capsule of *Anthoceros* and most Mosses).

XI. Differentiation of tissues is fairly advanced in the seta of some Mosses.

Now under the above heads are included those features which, if collectively present in one organism, might lead to the production of such more elaborate forms of shoot as are seen in the lower vascular plants, except two, and they are prominent ones, viz. :—

I. The formation of appendicular organs.

II. The subdivision of the archesporial layer to form isolated patches, instead of one continuous tissue.

With regard to the former—the origin of appendicular organs—we are still in the dark, though various suggestions have been made. In approaching this difficult question, I wish it to be understood that the criticisms and suggestions now to be made are offered only in the most tentative way, while it will remain to be seen how far they may be supported by the developmental facts to be described below.

Most of the hypotheses hitherto advanced have been based upon the belief that it is in the Filicineous series that the nearest point of contact between living Vascular Cryptogams and Bryophyta is to be found. For instance, KIENITZ-GERLOFF ('Bot. Zeit.,' 1878, p. 55), in putting forward his suggestion that the head of the

Moss-sporogonium is foliar in its nature, while the axis is rudimentary and is represented only by one quadrant of the upper half of the zygote, which later takes part in the formation of the foot, draws his detailed comparison with the Fern-leaf (pp. 55–56). Again, PRANTL (' Unters. z. Morph. d. Gefässkrypt.,' Heft 1, p. 62, &c.) discussed the matter from the point of view of a comparison between Moss-fruits and the simplest imaginable Fern plant. LEITGEB was, however, more cautious (*loc. cit.,* Heft 6, p. 61), though he seems to have had comparatively large-leaved forms in his mind when he wrote as follows :—" I believe that this much is certain : if we would trace the spore-forming generation of the Vascular Cryptogams from Liverwort-sporogonia, we must designate the cotyledons as the homologues of the latter, upon which at first the spore-formation must have been carried out, until, when the embryonic branching, *i.e.,* the formation of the stem and its lateral members (leaves) took place, it passed over to the latter."

Very much the same view was entertained by NAEGELI (' Abstammungslehre,' p. 476). Starting from the consideration of the steps of advance of the sporogonium of Liverworts, and distinction of a vegetative base from the sporogonial head, he discussed the mode of elaboration of the latter, suggesting it as possible that the apex became vegetative, "and that the sporogonium developed as a lateral outgrowth on the apex ; further that this sporogonium by further modification . . . . developed into the leaf-bearing stem." But he contemplated it also as possible " that the thallome-like sporogonium, as it became vegetative, may have extended directly (without a preliminary lateral branching) to the leafy stem." He further recalls the fact that branching occasionally occurs in Moss-sporogonia, and suggests that this may have recurred repeatedly, in connection with continued apical growth of the whole. "With the continued growth of the sporogonium in length, the spore-producing portion of it behind the apex is raised aloft, so that a stalked sporogonium is the result. The lateral branches are also fertile, and develop as sessile sporogonia. Thus a strobiloid sporogonial system arises which is either the direct continuation of the original thalloid body, or if, as was first assumed, a lateral sporogonium was produced by branching, a lateral continuation of it" (p. 477). He then suggests how the sporogonia may have increased in size, and partially passed over to the vegetative state, their main body becoming leaf-like, while the spore-production is limited to a part of them : the result would be a leafy axis, of which all the leaves would be sporophylls, a condition not unlike that of *Lycopodium.* The idea of NAEGELI (as embodied in his 'Abstammungslehre,' pp. 475–479) refers the elaboration of the sporogonium so as to form the leafy shoot to some form of *branching :* in this it does not differ from the other theories above referred to ; in all of them the origin of foliar members has been imagined as some process comparable to such branching of sporogonia, as is sometimes seen in abnormal cases. *In all of them the apex of a sporophyll is assumed to represent the apex of a sporogonium, or of some branch of a sporogonium.* I think that the origin of this idea is to be found in the assumption so often made that the

nearest point of contact with the Bryophyta is to be sought among the Ferns. Now the Filicineous leaf, even in its simplest forms, is a highly organized foliar structure: in many ways the leaves of Ferns are peculiar, while in their higher developments they attain such complexity as is hardly equalled in the vegetable kingdom. It appears to me improbable that even the simpler types of the Filicineous leaf will serve as indications of the origin of the foliar development exhibited in other vascular plants. I think it is rather among those series of homosporous Pteridophyta, such as the Lycopodineæ and Equisetineæ, in which a simpler type of leaf is the rule, that the inquiry may be pursued with better prospect of success.

Now the Lycopodineæ and Equisetineæ are *strobiloid* types, in which the vegetative leaves and sporophylls are relatively small, and the axis relatively large; while the leaves are closely associated together in large numbers on the axis. The sporophylls usually bear but few sporangia, and neither vegetative leaves nor sporophylls show localized or continued apical growth in any marked degree. It has become the practice in recent years to treat the shoot—whether sterile or fertile—as a whole (SACHS 'Lectures,' p. 3), of which the axis and leaves are component parts. This thoroughly sound practice is more obviously advantageous in cases where the axis is relatively large, and the leaves relatively small and crowded, and it will assist us in our discussion of these strobiloid Pteridophyta.

The theories of origin of foliar members above cited, except perhaps NAEGELI's, assumed that the apex of the sporogonium represents the apex of the coming sporophyll, while the axis originated either as a somewhat similar branch, or as a lateral bud in some way not always strictly defined. As the result of comparative study of Bryophyta and Vascular Cryptogams, I would now put forward as a working hypothesis, *that in the strobiloid Pteridophyta the apex of the sporogonium is the correlative of the apex of the strobilus.* The result of this would be that *the appendicular organs would all have been of lateral origin,* appearing as an eruption of outgrowths, probably unimportant at first, from the surface of the relatively preponderating axis. Such branching of the sporogonium as occasionally is seen would find its correlative, not in the formation of leaves, but in the terminal branching of the shoot or strobilus, as in *Lycopodium,* or rarely in the strobilus of *Equisetum* ("acrogene Verzweigung," NAEGELI, 1, *loc. cit.,* p. 478). As in the higher Bryophyta, so in the strobiloid Pteridophyta, a distinction is commonly found between vegetative and spore-bearing regions of the sporophyte; the seta of the former may then be the correlative of the vegetative region of the latter, while *the sporogonial head would correspond to the whole strobilus.*

It would be premature to discuss details of origin of the appendicular organs, but on the view above sketched, it would appear possible that these should originate, not only from the sporogonial head (as sporophylls), but also from the sterile region (as vegetative leaves), and these two types of foliar organs may have been produced independently of

one another, while a further addition to the vegetative region might be made by sterilization of sporophylls. (See below, p. 535, &c.)

I only know of one previous suggestion of such a nature as the above; it was made by the late Mr. VAIZEY, in a very tentative way ('Roy. Soc. Proc.,' vol. 45, p. 151), in connection with his work on the apophysis of *Splachnum luteum*. He wrote as follows: "That the apophysis performs the functions of a leaf, and is, therefore, *analogous* with the leaves of vascular plants, I think there can now be no doubt, and as this structure is a development of the sporophyte, the possibility of its being also *homologous*, either directly or indirectly, suggests itself. I am myself inclined to believe that the two are homologous." While admitting the analogy thus brought forward, I am not prepared to contend for the actual homology of the apophysis with a leaf or leaf-sheath. But I think we should do wrongly if we neglect to recognize that there is here, at least, a suggestion how a foliar development such as that of *Equisetum* may have taken its origin. If such sheaths were produced in large numbers in the lower vegetative region of a sporogonium, something like the simple shoot of *Equisetum* might be the result. A similar development in the sporogonial head might produce the strobilus. This view would harmonize readily with the embryology of *Equisetum* as described by SADEBECK, 'Pringsh. Jahrb.,' vol. 11; and by BUCHTIEN 'Bibliotheca Botanica,' No. 8, 1887.

But such development as this would involve the second prominent feature which we above noted as wanting among the Bryophyta (above, p. 491), viz., (2) the subdivision of the archesporial layer to form isolated patches, instead of one continuous tissue. Is there any reason to think such a subdivision probable, and how could it take place? To answer this question recourse must be had to comparison of certain details of spore-production in the Bryophyta, and in the Vascular Cryptogams; the former are already provided by the researches of LEITGEB, while I hope to add to the latter in subsequent pages. The full discussion of this subject must, therefore, come at the end of this memoir; but in the meanwhile certain facts and conclusions from study of the Bryophyta may be considered. It has been seen that sterile cells (elaters) in certain Liverworts appear to be massed together, and form solid tissues, as in the case of the columella; it is, therefore, reasonable to expect that some similar process may occur again in such Liverworts, or other plants, as show sterile cells scattered through their sporogenous masses. Now the *Anthoceroteae* have by various writers been recognized as plants whose sporophyte approaches that of vascular plants more than that of other Bryophyta (LEITGEB, *loc. cit.*, Heft 6, p. 60; PRANTL, *loc. cit.*, Heft 1, p. 62). In these plants a large proportion of the cells produced from the archesporium are sterile, and it would thus appear that a formation of sterile septa might be expected to result from a process of massing of these, such as is shown by LEITGEB to have produced the columella. But while noting this, I would not be understood to use it as more than an illustration, for I do not suppose that any living Bryophyte really represents the progenitors of vascular plants.

Thus we see that certain of the factors which might lead to the production of a more complex type of sporophyte are represented in living Bryophytes.

The next step will be, bearing these facts in mind, to inquire whether on the other side of the gulf, *i.e.*, among the lower vascular plants, characters of a similar nature are to be observed, and we shall look for evidence in answer to the following questions :—

(1.) Are sterile cells distributed among the sporogenous cells in any Vascular Cryptogams ?

(2.) Do any Vascular Cryptogams show a distinct part which may be correlated in position, structure, development, and function with the sporogonial head of the Bryophyta ?

(3.) Are the sporangia ever borne in such relation to one another as to suggest a common origin by sub-division of simpler parts ?

(4.) Is there direct evidence in any Vascular Cryptogams of conversion of potential sporogenous tissue into masses of sterile tissue, or conversely of conversion of sterile septa into spore-producing cells ?

I am quite aware that, even if all these questions were answered by abundant evidence in the affirmative, this would not afford complete proof of the mode of descent of Vascular Cryptogams from simpler Bryophytic forms. Few biological hypotheses are completely proved, or even susceptible of proof, under the present order of nature. But what I submit is, that the search for answers to such questions as the above may, in the light of the facts already derived from the study of the Bryophyta, go far to support the working hypothesis above sketched in its broad outlines, and raise it to the position of a reasonably probable theory.

EQUISETUM.

The first detailed account of the development of the strobilus and sporangia of *Equisetum* was given by HOFMEISTER for *E. arvense* ("Higher Cryptogamia," 'Ray Soc.,' p. 280, Pl. 36); his results were in the main adopted and verified by RUSSOW ('Vergl. Unters.,' p. 147, and Pl. 9), but, as regards the origin and early history of the sporangium, the investigations of the latter add little to the facts already acquired by HOFMEISTER. A point of difference between these two writers is in the reference of the sporangium, as regards its origin, to a single parent cell. HOFMEISTER speaks of the shields (sporangiophores) exhibiting "at five or six points a rapid cell-multiplication, produced by the division of one of the cells of the under surface of the shield," and he appears to refer the whole of each sporangium to *one* original cell. It will be seen that the essential part of the sporangium, viz., the sporogenous tissue, is referable in most cases with certainty to the successive segmentations of a single original cell, but the sporangium as a whole is of the eusporangiate type ; this was recognized by RUSSOW, and does not now admit of any doubt.

The most accurate examination of the sporangiophores and sporangia of *Equisetum*

hitherto made has been by GOEBEL, his illustrations being taken from *E. limosum* ('Bot. Zeit.,' 1880, p. 549, Pl. 8, figs. 2–6; also, 1881, Pl. 6, fig. 1). The two most important of these are quoted in his 'Grundzüge,' as fig. 223A and 223B (but under the name of *E. palustre*); in these figures, the archesporial cells are shaded so as to distinguish them. It has always been a difficulty to me to accept the story of development, as stated by GOEBEL; the earlier stage ('Bot. Zeit.,' 1881, Pl. 6, fig. 1, quoted as 'Grundzüge,' fig. 223A) shows an archesporium consisting of only one cell, which is covered externally by two relatively deep cells, one of which is superficial; the later stage (fig. 223B), shows the sporogenous cells, eight in number, covered at the apex by three relatively shallow cells, which do not together measure a greater depth than the one superficial cell of the earlier figure. Assuming the figures to be drawn under the same magnifying power (on which point I find no statement in the original paper, or in the 'Grundzüge'), it would seem either that the cells overlying the archesporium contract and become shallower as development proceeds, or that the recognition of the archesporium as a single cell in the earlier stage (fig. 223A) is at fault. Moreover, the history of development, as illustrated by the figures, is far from complete. In order to give a satisfactory account of so bulky a structure, it should be cut in three directions, radially, tangentially, and transversely, at different stages, but this was not done by GOEBEL. It thus appears that there is need of a re-examination of development of the sporangia of *Equisetum*.

Equisetum arvense L. (figs. 1–9).

The results yielded by a study of *E. arvense* have afforded a more complete account of the early phases of development than in other species, and they will be described first. *E. limosum* has also been examined for purposes of comparison with GOEBEL's results and with the data from *E. arvense*. It will be seen that the details in the latter species are liable to considerable variation, and differ in some points from the more regular *E. arvense*.

Radial sections through the very young strobilus of the last-named species show that the sporangiophores arise as already described by HOFMEISTER, RUSSOW, and GOEBEL; they appear as ring-like outgrowths, which, in the longitudinal section, involve a considerable number of cells, with a fan-like tracery. Comparing those of *E. arvense* (fig. 1) with those of *E. limosum* (see GOEBEL, 'Bot. Zeit.,' 1880, Plate 8, fig. 2), the former are of a more bulky character, and this will be found the case also with the sporangia of the species. As in *E. limosum*, the tissues of the sporangiophore, when cut in radial section, are referable to the outgrowth of six cells, but many more anticlinal divisions appear in these before the convex outgrowth is considerable, than is the case in *E. limosum* (compare GOEBEL, 'Bot. Zeit.,' 1880, Plate 8, fig. 2). Accordingly the sporangiophore of *E. arvense* is from the first of a more massive type than in *E. limosum*. In the larger sporangiophore shown in fig. 1 it is believed that the

cells marked (⊙) are correctly recognized as those which will give rise to the essential parts of two sporangia; it will be observed that these cells occupy corresponding positions on either side of the sporangiophore, adjoining the anticlinals 1 and 5. As the sporangiophores grow older the anticlinals become more strongly curved, owing to the more active growth of the central parts, while the sporangiophores of successive series coming in contact press upon one another, and their sides become flattened. Growth of the future sporangia now becomes more rapid, so that they begin to project (fig. 2), while cell-divisions rapidly succeed one another. The succession and exact position of their walls is liable to some variation, as is almost always the case in the development of large cell-masses. The description now given is that which is found to be most typical, and it will be seen that a single cell is the ultimate parent of the essential parts of the sporangium; such a cell is shown, still undivided, in fig. 1, adjoining the anticlinal 5, in the larger sporangiophore. The first division in this cell is by a periclinal wall (fig. 1) separating an inner cell (shaded) from an outer one; the inner cell is divided first by anticlinal walls at right angles to one another (compare figs. 2, 3, and 7) into four, and subsequently by periclinal walls into eight cells, which form the lower part of the sporogenous tissue. But this is not the whole of the tissue from which spores are formed; a comparison of older stages (figs. 3, 4, 5, 6) shows that, by subdivision of the outer of the two original cells, shown in fig. 1, additions are made to the sporogenous tissue; these appear in the following way. The outer cell usually divides by crossed anticlinal walls (figs. 2, 3, 7, and 8), which are succeeded by periclinals (figs. 3, 7); the inner cells thus formed are added to the sporogenous tissue, while the outer form the apical part of the wall of the sporangium and the tapetum; a careful comparison of figs. 1–7 will show that the sporogenous tissue is not limited by the first periclinal wall, but that cells derived from part of the outer of the two original cells form an essential part of the sporogenous mass; these cells are marked with a cross (×) in all the figures. In order to obtain tangential sections, which shall traverse the axis of the obliquely placed sporangium, the direction of section must be slightly inclined; such a section is seen in fig. 7, in which the cell-tracery corresponds in all essential points to that of fig. 4; it further demonstrates how slight as yet is the projection of the individual sporangia beyond the margin of the sporangiophore, as is shown by the gentle curve of the surface above the apex of the sporangium.

Owing to this very slight projection of the young sporangium, and its oblique position, transverse sections are not easily obtained in the very young state; but when the sporangium has attained the size and development of fig. 6, a transverse section shows plainly enough the relations of its parts (fig. 9); the sporogenous tissue which is shaded is readily referable in its origin to the segmentation of a single cell of the transverse section; certain cells of the adjoining tissue develop of large size, and with dense protoplasmic contents, and it has been difficult to decide whether there be not also an addition to the sporogenous tissues from such lateral cells; some longi-

tudinal sections appear to support this idea, but it has never been possible to prove beyond doubt that any such additions to the sporogenous tissue really take place; however, of the additions from segmentation of the outer of the two original cells (fig. 1) there cannot be any question after comparison of figs. 1–8.

It is interesting to compare these results with Russow's drawings ('Vergl. Unters.,' Plate 9); his fig. 179 shows on the left side a sporangium of age intermediate between my figs. 1 and 2, and with virtually the same structure; while his fig. 177 corresponds substantially, though not in the minute details, with my fig. 3. Such correspondence, though not surprising seeing that the drawings refer to the same species (*E. arvense*), is certainly satisfactory.

After the sporogenous tissue has been thus laid down, the cells which lie above it divide by periclinal and anticlinal walls; some four or five layers of cells are thus formed (fig. 6), of which the outermost develops as the apical part of the wall of the sporangium, the rest of the wall being contributed by the superficial cells of the lateral parts of the sporangium; the cells directly surrounding the sporogenous mass assume the characters of a tapetum, while the intermediate cells become gradually compressed and finally disappear without any special modifications, as the period of maturity of the sporangium approaches. These later phases of the development having been more carefully followed in *E. limosum*, we may now proceed to examine the results of investigation of that species.

<center>*Equisetum limosum*, L. (figs. 10–21).</center>

It has been already shown that Goebel's account of the development in this species leaves some points uncertain; this is probably due in part to the very considerable variations of detail of the sporangia even in the same strobilus; how great those variations may be is shown by fig. 10, which represents three sporangia in juxtaposition with one another (*a*, *b*, *c*), together with the stalks of the sporangiophores which bore them (*sp.*); not only is there a great difference in size and outline of these sporangia, but also in the number of the sporogenous cells as seen in the transverse section, and even in the number of layers of cells composing the wall. Such variations of detail naturally increase the difficulties of tracing the course of development.

The earliest stages of growth have not been seen in *E. limosum*; but this is immaterial after the observations on *E. arvense*, and in face of Goebel's drawing ('Bot. Zeit.,' 1881, Pl. 6, fig. 1); for there is no reason to doubt the correctness of the cell-net there shown, in what is obviously a median section. The only question is the correctness of recognition of the archesporium: it will be seen from a study of older sections that the second cell, immediately overlying the shaded archesporial cell of Goebel's figure, should also be reckoned a part of the archesporium; as in

E. arvense, this cell is added to the first archesporial cell, and originates by a subsequent periclinal segmentation of the superficial cell.

The sporangia of *E. limosum* are usually less bulky than those of *E. arvense* : their apex is commonly more pointed, and the archesporial series may remain for a considerable time undivided by longitudinal walls : this is suggested by Goebel's figure ('Bot. Zeit.,' 1880, Pl. 8, fig. 3), and is conspicuously the case with fig. 11, in which the archesporium consists of only a single row of three cells ; such an appearance would be presented by the sporangium (*a*) fig. 10, if cut through radially. Other sporangia show the archesporium as a double row (as in fig. 12); it is to be noted here that the apical limits of the two rows do not exactly coincide, the row next the sporangiophore being longer than that more remote from it. Lastly, the sporangium shown in fig. 13 resembles more nearly the type of *E. arvense,* as regards the regularity of segmentation of the apical part of its sporogenous tissue, though the basal is irregular ; it also has a rounded apex ; the cells marked (?), adjoining the sporogenous tissue laterally, are of large size, and have dense protoplasm ; it is possible that they may be lateral additions to the sporogenous tissue, but such additions have not been definitely proved to take place. Of older sporangia cut radially, two extreme examples have been drawn ; of these, fig. 14 is of the more pointed type, and comes from near the apex of the strobilus ; it will be noted that the limits of the sporogenous tissue on either side of the median line are unequal, and that, as in fig. 12, the sporogenous tissue is more extensive on the side next the sporangiophore than on that which is more remote ; the result is a very irregular disposition of the tissues towards the apex. The other example (fig. 15) shows a very bulky sporangium with broad rounded apex : this comes from the lowest tier of sporangiophores, and directly adjoins the annulus. The segmentation has here been very regular, and conforms more nearly to that of *E. arvense* than does that of the large majority of the sporangia of *E. limosum.*

Of the tangential sections those shown in figs. 16, 17, 18 were from the same strobilus as the radial sections, figs. 11, 12, 13. Fig. 16 represents one of the simpler types of sporangium, in which the sporogenous tissue consists in part of only a single row of cells. Figs. 17 and 18 are of a more bulky type, especially the latter, while, in addition to the sporogenous tissue which is shaded, the cells marked (?) are of considerable size and contain dense protoplasm ; it is here again a matter of doubt whether cells of other genetic rows are not added laterally to the sporogenous tissue derived from the median row. Fig. 19 represents a rather older sporangium of the more bulky type, from the same strobilus as those drawn in transverse section in fig. 10, and in longitudinal section in figs. 14 and 15 ; it shows a sporogenous tissue of even a more massive character than that of *b* (fig. 10), and corresponds probably most nearly in complexity of structure to the sporangium drawn in fig. 15.

The whole of these drawings serve to illustrate the considerable fluctuations of bulk and complexity of structure in the sporangia of this species, and this is quite in accord

with the experience of GOEBEL ('Bot. Zeit.,' 1880, p. 551). The observations here detailed agree with his so far as regards the reference of the sporogenous tissue to a single axile row of cells, with reservations of some questionable cases where parts of lateral rows may contribute a few cells ; but this has been actually demonstrated in no single case. I cannot, however, agree with him in referring the whole sporogenous tissue to a single cell of that row ; here, as in *E. arvense*, additions are made to the primary archesporium by development of more superficial cells (marked $\times$ in figs. 12, 13, and 16, where they are clearly distinguished). The cells of the axile row sooner or later divide by longitudinal walls, and again subdivide in various directions so as to produce a sporogenous tissue, which, in longitudinal section, would appear to average about 72 cells.

Turning now to the cells which surround the sporogenous tissue, their arrangement is rather irregular, and the band of superficial tissue is usually thicker at the apical and lateral parts than on the anterior and posterior faces (figs. 10, 14) ; the sporogenous tissue is covered at the apex by three to five layers of cells, which are narrow, and are arranged with some approach to regularity. Of the tissues composing this peripheral band, the innermost row of cells develop as the tapetum, dividing by repeated anticlinal walls, and may thus be distinguished from the rest (fig. 20) ; the outermost series become enlarged and their walls strengthened, forming the permanent wall of the sporangium, while the cells which intervene are compressed, and their nutritive substances withdrawn as the sporangium approaches maturity.

As in other cases, the cells of the sporogenous tissue separate, round themselves off, and divide into tetrads. This process has been repeatedly the subject of detailed observation, and I do not propose to follow it further ; there is, however, a fact of some importance which does not appear to have been noted by other observers, that is, the partial sterilization of the sporogenous tissue. It has been remarked above that an average number of sporogenous cells in a longitudinal section, before they separate and round themselves off, is 72, but the average number of these cells, as seen in one section after they are rounded off, is only about 44 : the explanation of this reduction is to be found in the fact that a considerable number of cells become disorganized during the process, as is shown in fig. 21. Here it is seen that the tapetum is still a continuous band outside the sporogenous cells, though the cell-walls of the tapetum are disorganized, and the nuclei float freely in the continuous protoplasm. The outer sporogenous cells still form an external barrier, but certain cells of the sporogenous mass have lost their definite outline, their protoplasm has become irregular, and their nuclei have lost size and brilliancy, and differ materially from those of the rounded sporogenous cells : the number of cells thus altered in the small part drawn is 13, as against 30 which are rounded off. These numbers, rough though they are, will suffice to explain the discrepancy above noted. In fact, about one-third to one-quarter of the sporogenous cells are disorganized and do not form spores : their function is that of a diffused tapetum, and there can be no doubt that their substance

contributes to the nutrition of the survivors. Ultimately the external tapetum makes its way between the rounded sporogenous cells, and then it is no longer possible to distinguish the nuclei and plasma of the external tapetum from those of the disorganized sporogenous cells. It will subsequently be shown that a similar breaking down of sporogenous cells takes place in other types of Vascular Cryptogams, and it must be recognized as one of the forms which *sterilization* of this tissue may be found to assume.

Results from Study of Equisetum.

1. The sporangium is Eusporangiate.

2. The essential parts of the sporangium, that is, the contents, are ultimately referable in origin to a single superficial cell.

3. The first division of this cell is periclinal : *the inner cell forms only part of the sporogenous tissue, to which addition is made of other cells derived by further segmentation of the outer cell.* The archesporium of *Equisetum* is thus shown to be not a single hypodermal cell in the sense laid down by GOEBEL

4. The tapetum is derived from the series of cells immediately surrounding the sporogenous mass.

5. *About one-third of the cells of the sporogenous mass are broken down and disorganized without forming spores ;* these serve physiologically as a diffused tapetum, and help to nourish the developing spores.

6. The superficial cells form the wall of the sporangium, while those between the tapetum and the wall are pressed out of shape and disorganized as development proceeds.

7. The sporangiophores and sporangia of *Equisetum arvense* when young are, on the average, of a more massive type and more regular segmentation than those of *E. palustre ;* and it is to be noted that the former is a terrestrial, the latter an aquatic species.

Theoretical Consideration of the Results.

Perhaps the most interesting point of detail in the results thus stated is that the archesporium of *Equisetum* is not, strictly speaking, of hypodermal origin, but that an addition is made to the first hypodermal archesporial cell by further segmentation of the superficial one. In this, it will be subsequently shown that *Equisetum* does not stand alone. In *Isoetes*, also, and in *Selaginella*, and, in rare cases, in *Lycopodium*, similar additions appear to be made to the sporogenous cells first laid down ; the cells thus added are shown in *Equisetum* to develop into spores, and are thus archesporial in their character. It will be seen that these facts will affect the generalization of GOEBEL, as given in the paper in which he introduced the idea of the archesporium ('Bot, Zeit.,' 1880, p. 569). He there states "that in all the Vascular Cryptogams

which he investigated there is a hypodermal archesporium." GOEBEL assigns no lateral limit to the archesporial tissue as applicable for all cases, for, according to his description, the archesporium may be a single cell, a row of cells, or even a layer or sheet of cells (*loc. cit.*, p. 564). This my observations, to be detailed below, amply confirm. The only definite limit which GOEBEL assigns for all Vascular Cryptogams which he investigated is that of depth of origin; they are all hypodermal. In the strict sense, however, this is not the case in *Equisetum*. Accordingly, I do not see how any strict topographical definition can be applied to the archesporium which shall hold for all Vascular Cryptogams. It is true that in most Vascular Cryptogams, and probably in all, the archesporium is ultimately referable in its origin to the segmentation of one or more superficial cells. But what part of the shoot in these plants does not originate in this way? A statement of this fact would be in no way distinctive in plants in which one or more initial cells are commonly the ultimate parents of the tissues of all parts, whether deeply seated or superficial. *While the results of my observations on sporangia of Vascular Cryptogams will confirm GOEBEL's statement that the sporogenous tissues are referable in origin to a definite cell or cells (archesporial cells), I find it impossible to give to these cells a strict topographical definition which shall apply in all cases.*

This conclusion need be in no way surprising, for it is plain, in the first place, that the segmentation which gives rise to the archesporium, in Vascular Cryptogams, is different from that in Phanerogams; in the latter the dermatogen is as a rule definite, and distinct in origin and segmentation from the subjacent hypodermal cells, which ultimately give rise to the sporogenous tissue.

In the second place the origin of the archesporium in the Musci is not uniform; for it has been shown by WALDNER ('Die Entw. d. Sporogone v. *Andræa* u. *Sphagnum.*' Leipzig, 1887), that while in *Sphagnum* the archesporium is formed from the endothecium, in *Andræa* it is derived from the amphithecium, the latter is also the case for other Mosses.

The case of the Anthocoroteæ has also been noted above (p. 489); in these plants there is absence of uniformity in origin of the sporogenous tissue, within the limits of the family. This I take to be a most instructive example in connection with the present question. While there is, thus, want of uniformity within the Bryophyta in the depth of origin of the archesporium, there is also a difference in its mode of origin as we pass from the Bryophyta to the Vascular Cryptogams, and again, in point of segmentation, as we pass from the latter to the Phanerogams. In view of these variations it need be no matter for surprise that in the Vascular Cryptogams themselves the mode of origin of the archesporium is not susceptible of a strict topographical definition which shall apply for all cases.

Equisetum being a strobiloid type, in accordance with our working hypothesis, we shall look upon the strobilus as the counterpart of a sporogonial head, while the apex of the strobilus will then correspond to the apex of a sporogonium (see p. 492).

As regards the segmentation of the apex I see no difficulty in the present case, for the apical cone of *Equisetum*, as seen in the strobilus, is in many respects similar to that of certain sporogonia of Bryophytes ; few would see in the difference between a two-sided and a three-sided cell, or between a three-sided pyramid and a growth with more than one initial, a serious objection to such a comparison as I suggest.

The sterile central part of the strobilus, with its vascular bundles, would be the counterpart of a columella. It is, doubtless, more bulky, and its structure more complex, but, as regards its topographical relation to the spore-production, it is undoubtedly similar to the columella of Bryophytes. Now, among the Anthoceroteæ, there is found great variation in the bulk of the columella ; in the genus *Notothylas*, especially, such variations are seen. I accordingly look upon the relatively greater bulk of the central sterile mass in *Equisetum* as no obstacle to its recognition as the counterpart of the columella.

The form of the very young strobilus is clearly like that of a sporogonial head ; the chief difference lies in the simplicity of external surface of the Bryophytic sporogonium and its connected archesporium, while, in *Equisetum*, the surface produces outgrowths (the sporangiophores), and the archesporia are isolated. But the appearance presented during development is very suggestive, and may with propriety be thought to give a clue to the origin of the more complex structure. The young strobilus has first a smooth surface, on which undulating swellings appear, as already noted by HOFMEISTER and by GOEBEL. At an early stage, the sporangia may be recognized as originating at certain points on these outgrowths (compare figs. 1–4, Pl. 42), certain superficial cells, by their segmentation, supplying the essential parts of the sporangia. On our hypothesis, we should see in these cells and the products of their segmentation, the isolated remains of a largely-sterilized potential archesporium, while the fact that they are carried out upon the massive sporangiophores need in no way disturb this comparison of the archesporial cells themselves. It may, however, be objected that, in *Equisetum, superficial* cells provide the essential parts of the sporangia ; but this again is no real difficulty, for, ultimately, the same is the case in many Bryophytes ; the amphithecium is, in the young state, a series of superficial cells, and it is this layer which in many cases yields the sporogenous tissue. But, in this connection, the absence of strict uniformity of origin of the archesporium in the Bryophyta is specially important. The gradual appearance of the columella, and relegation of spore-production to more superficial tissues is illustrated in the Anthoceroteæ ; if such a modification were carried out but a little further, if the columella were more bulky, and the definite specialization of archesporia deferred, the result, *as regards locality*, would be such as is seen in *Equisetum*. There is, however, the essential difference of the archesporium being in the Bryophyta one continuous band, in *Equisetum* isolated patches ; at present, we have no direct evidence before us how such a change took place, but, referring again to the analogy of the Anthoceroteæ, the origin of the columella, by grouping of sterile cells, has been concluded by LEITGEB (see p. 489).

We see that, in *Anthoceros*, a large proportion of the cells resulting from the arche-sporium are developed as sterile cells; a grouping of these together might produce such a condition as is seen in *Equisetum*, viz., the isolation of those cells which still remain fertile (archesporia), while between them extensive tracts of sterile tissue would intervene. Such would be an extreme example of the septate condition, and it will remain to inquire whether any evidence is to be found among other Vascular Cryptogams as to the origin of sterile septa from a continuous potential archesporium. In the meanwhile, we may note with additional interest the presence of a large number of sterile cells in the young sporangia of *Equisetum*, and, though they do not develop as elaters with thick walls, still in their condition of sterility they may pro-perly be compared with those of the Liverworts, especially of the type shown in *Oxymitra*.

Another question will be whether from other types of Vascular Cryptogams evidence can be gained of the origin of upgrowths (sporangiophores) which would raise the sporangia beyond the surface of the part on which they are produced; such evidence would be of peculiar value in its bearings upon the question under discussion.

In any discussion such as that before us, the facts concerning the strobili of certain fossil forms will have to be considered. Whatever opinions may be held as to the nearer or more remote kinship of modern *Equiseta* to the Calamarieæ, there can, I think, be little doubt that the strobilus of a modern *Equisetum* as a whole is the homologue of the whole spike-like fertile branch in the latter group. Assuming this to be granted, we see on comparing these several strobili that there is some variety in their construc-tion, and in the relations of the component parts one to another. Such points will have to be considered in the general discussion of the question, which will be taken up later.

In the meanwhile I see in the case of *Equisetum* no *à priori* objection to our working hypothesis; the details of development would rather support it. But before it can be seriously entertained, some more consecutive evidence will be required that formation of sterile masses of tissue from a potential archesporium does occur; we shall also inquire whether it may be concluded, on comparative grounds, that septa have been produced within any naturally allied series of Vascular Cryptogams, thus dividing into separate parts an originally continuous archesporium.

LYCOPODINEÆ.

The Lycopodineæ are also strobiloid forms, to which our working hypothesis may be applied. In examining them, we may be prepared to see in the strobilus the counterpart of a sporogonial head, while certain of the vegetative parts may have resulted from further development of a sterile part corresponding to a seta. In the latter category may, perhaps, be placed the protocorm of TREUB, with the leaves (protophylls) which it bears; it will, however, be shown that the majority of the

sterile leaves of certain Lycopods are more probably to be looked upon as sterilized sporophylls. These ideas will be applied in the study of various Lycopodinous forms which will now be taken up.

PHYLLOGLOSSUM DRUMMONDII, KUNZE (figs. 22–33 and fig. 92, *a*, *b*, *c*.).

The vegetative organs of *Phylloglossum* are now fairly well known, both as regards external form and internal structure. The earlier observations of METTENIUS* have been extended by M. BERTRAND,† who investigated chiefly the mature structure, and by myself as regards the germination of the tuber, and the formation of the new vegetative parts from it.‡ These two researches, conducted separately and published almost simultaneously, led to virtually the same conclusion, viz., that we may regard *Phylloglossum* as a form which retains, and repeats in its sporophyte generation, the more prominent characteristics of the embryo as seen in *Lycopodium cernuum*. This conclusion was based on comparison of *Phylloglossum* with young stages of development of *Lycopodium cernuum*, as described by Dr. TREUB in his earlier memoir on that plant ;§ but the similarity in certain points is now seen to be even more striking since the publication of this author's more recent observations on the embryology of this plant ; || while assenting to the general conclusion, Dr. TREUB prefers to give the thesis a slightly different form, by inverting it, and asserting rather that in their young state *Lycopodium cernuum*, *inundatum*, and *salakense* repeat the *Phylloglossum*. In view of Dr. TREUB's theory of the *Protocorm*, this amendment may be accepted. He states the theory as follows :--¶

" The embryonic tubercle in the Lycopods is *a rudimentary organ.*" " The organ theoretically admitted as occurring in the ancestors of actual Vascular Cryptogams, and styled above ' The predecessor of the leafy shoot as it is now seen in the genus *Lycopodium*,' exists even at the present day as a transitory stage in the genus *Lycopodium*. This *organ is none other than the embryonic tubercle.*" Further, " I propose to give the embryonic tubercle of the Lycopodiums the name of the *Protocorm*." Later, referring to *Phylloglossum*, Dr. TREUB says : " it will be superfluous to say that, to me, the tubercles of *Phylloglossum Drummondii* are to be looked upon as protocorms, playing still a real and considerable part. The protocorm of multiplication of *Phylloglossum* has become a much more specialized organ than in the Lycopodiums."

From the above quotations, which put the whole matter on a firmer footing than

* ' Bot. Zeit.,' 1867.
† ' Arch. Bot. du Nord de la France,' 1885, p. 70.
‡ ' Phil. Trans.,' 1885.
§ " Études sur les Lycopodiacées," ' Ann. du Jard. Bot. de Buitenzorg,' 4, p. 107.
|| ' Ann. du Jard. Bot. de Buitenzorg,' 8, p. 1.
¶ *Loc. cit.*, 8, p. 30.

before, it appears that Dr. TREUB would endorse the last words of my former memoir on *Phylloglossum*, viz., that "it is a permanently embryonic form of Lycopod"; in fact, as he states,* *Phylloglossum* "would represent a more ancient group than that represented by living Lycopodiums."

This view, however, is not universally admitted.   M. BERTRAND, while recognizing the similarity between the early stages of *Lycopodium cernuum* and the adult *Phylloglossum*, remarks "It is singular, in the present case, that *Phylloglossum* is superior to the Lycopods, and still that its adult state is but a reproduction of an embryonic stage of the latter.   Here is a case of retrogression, which has as its prime cause the semi-aquatic habit."   I confess, with all respect to M. BERTRAND, that the anatomical characters on which he chiefly relies for assigning relative positions to the Lycopods do not seem to me a sufficient reason for placing *Phylloglossum* higher in the scale than *Lycopodium*.   As to the semi-aquatic habit, it seems to me that it is precisely in such positions that the most interesting early forms of the sporophyte might on *a priori* grounds be expected to occur ; if, as I have suggested elsewhere, antithetic alteration be an adaptive development closely connected with the progression from water to the land, then the semiaquatic flora should include some of the earliest types of development of the sporophyte.†   Accordingly, I am disposed to look upon *Phylloglossum as relatively a primitive form*, and for this reason I shall give it the first place in the description which is to follow.

One of the most marked characteristics of *Phylloglossum*, and one by which it is distinguished from species of *Lycopodium*, is that the transition from the leaves borne by the protocorm to the sporophylls of the strobilus is usually a sudden one. The zone of intercalary growth, by the extension of which the strobilus is carried up, appears to be a natural limit between the vegetative leaves on the one hand, and the sporophylls on the other.   In one specimen, represented in fig. 22, a single sporo-phyll is seated at some distance below the rest of the strobilus ; it has, however, a sporangium in its axil, and is therefore to be looked upon as a sporophyll which was left behind in the course of intercalary growth ; but it is also of larger size and more succulent development than normal sporophylls, and may therefore be actually an intermediate step between the sporophyll and those leaves (protophylls) which are borne by the protocorm.   On the other hand, it is not unfrequently found that the last of the protophylls is smaller than the rest ; this was shown in figs. 18, 20, 25, and 27, of my former paper, and alluded to in the text on pp. 670, 671, and 675.‡ The position of these leaves is close to the base of the strobilus in those plants which bear sporangia ; these leaves, again, are probably transitional steps between the typical protophyll and the sporophyll ; they are, however, inconstant, both in form and occurrence, and commonly the transition is a sudden one.   I think it probable

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* *Loc. cit.*, p. 73.

† 'Annals of Botany,' vol. 4, p. 347, &c.

‡ 'Phil. Trans.,' 1885.

that the part described by M. BERTRAND under the name of the " organ of METTENIUS,"* is simply one or two rudimentary leaves of this nature. This was suggested by M. BERTRAND himself (*loc. cit.*, p. 197), but whereas he describes this organ as of constant occurrence, there was very little constancy in its appearance in the specimens which I have examined.

The strobilus itself is almost always simple, but one specimen, grown in the Glasgow Botanic Garden, shows a branching (fig. 23) into two unequal limbs. The plant was a weakly one, and neither of the strobili appeared well developed ; it may therefore be looked upon as an abnormality, though it is interesting for comparison with the branched strobili of various species of *Lycopodium*.

For comparison, on the one hand with the structure of the mature protophylls as described by M. BERTRAND† and by myself,‡ and on the other with the protophylls of *Lycopodium cernuum*, as described by M. TREUB,§ I have made drawings of young states of *Phylloglossum*. Fig. 24 shows a whole plant, such as that of fig. 8 of my former paper, in median longitudinal section, while fig. 25, i., ii., iii., are successive transverse sections of a single leaf. It is true that no distinctive characters are apparent from these sections, but the rarity and morphological importance of this plant justify the representation of such details, even though the bearing of them may not be obvious at the present moment.

Applying our working hypothesis to *Phylloglossum*, which on grounds above stated we recognize as a relatively primitive type, we should see in the protocorm the counterpart of the seta of Bryophytes. It is enlarged it is true, and bears appendicular organs (protophylls and roots), but these are distinctly primitive in their form ; the leaves are arranged in a rather irregular fashion, the roots are exogenous and the whole character of this part of the plant is such as to fall in with the hypothesis. The distinction between this vegetative region and the strobilus is unusually definite, more so than in other Lycopods, while there is an entire absence of that intermediate vegetative region which forms the greater part of the plant of *Lycopodium*, even in such species as still show the characters of a protocorm in their embryonic stages. In the strobilus we should see the counterpart of the sporogonial head ; as in the case of *Equisetum*, it is more complex in external form than the sporogonial head, bearing appendicular organs, and separate sporangia ; but the same arguments as have been used in the case of *Equisetum* will apply also here in meeting antecedent objections on these grounds to the comparison which I suggest. The detailed examination of the strobilus will now be proceeded with.

The strobilus has been described in minute detail by M. BERTRAND,‖ as regards

* ' Arch. Bot. du Nord de la France,' *loc. cit.*, p. 74.

† *Loc. cit.*, p. 174.

‡ ' Phil. Trans.,' 1885, p. 673, and figs. 36, 37.

§ ' Ann. d. Jard. Bot. d. Buitenzorg,' 8.

‖ *Loc. cit.*, pp. 120-131.

its mature structure, but developmental data are at present wanting. This lacuna I am able partially to fill, from observations on specimens reared from tubers in the Glasgow Garden. The youngest state of the sporangium seen in radial section is that shown in fig. 26. The section includes the apex of the strobilus, which is occupied by a conical initial cell; round this are segments disposed with some regularity, but it is difficult to refer the whole growing point in origin to this single initial. A single initial was not found either at the apex of the mature tuber, nor of the young one, and sufficient material was not at hand to decide the time of its appearance at the apex of the strobilus, or whether it be constant in occurrence, or in form. Below the extreme apex the youngest leaf (1, ii.) appears as a broadly convex outgrowth, without any single initial, while the next older sporophyll (s^1) is already far advanced, and bears in its axil the young sporangium. This is a multicellular outgrowth, of which the cells, as seen in this section, are referable in origin to a single parent. Segmentation has, however, gone so far that a single archesporial cell (a) is completely surrounded by others, and occupies a rather deeply-seated position. Fig. 27 shows another section through the same sporangium (which, as in *Lycopodium*, is a sausage-shaped outgrowth, and may thus be traversed by numerous planes of radial section). Here the segmentation is more regular, and the archesporium appears divided into four cells by very delicate walls, which the treatment of the previous section had probably made invisible. The cells below the archesporium are also showing the first signs of that activity of growth and division, which results in the formation of the stalk of the sporangium. Fig. 28 shows a rather later state with less regular segmentation, while in fig. 29, the first periclinal divisions are appearing in the cells of the wall of the sporangium. (N.B. This section is not exactly median.) Finally in fig. 30 is seen a sporangium in which all the essential parts are present. The enlarged head is borne upon a short and rather massive stalk, and it is bounded externally by a wall composed for the most part of two layers. Of these the inner would divide subsequently, and the inmost layer thus formed would be the tapetum. The basal part of the tapetum is already present as the irregular series of cells which adjoin the base of the sporogenous mass, the latter is already showing signs of the separation and rounding off of its cells preparatory to the formation of the tetrads. Taking all these facts together it is plain that the development of the sporangium of *Phylloglossum* corresponds in essential points with that of *Lycopodium Selago,* as laid down by GOEBEL,* or as described more in detail in later pages of this memoir (p. 511, &c.).

To gain an adequate knowledge of a solid body such as the sporangium of *Phylloglossum,* transverse and tangential sections must also be examined ; but, owing to the small quantity of material, the details could not be so completely worked out as they will be in some species of *Lycopodium* to be dealt with later. Transverse

* ' Bot. Zeit.,' 1880, p. 561.

sections through a sporangium of age corresponding to fig. 28, show that the archesporial cells form a series of which the number is at least four, and is probably larger (fig. 31) ; the section shows the sporangium united both with the stem and the sporophyll, the plane of section must therefore have been about the dotted line in fig. 28, and the two will mutually explain one another. Fig. 32 again shows part of an older sporangium in similar section, where the wall has already divided into two layers, while the sporogenous cells still form a continuous tissue, and have not yet completed the divisions before the separation of the spore-mother-cells.

Finally a tangential section (fig. 33) of a sporangium rather younger than that of fig. 32 shows the form of the sporogenous mass, which is curved in a sinuous fashion, while the cells are obviously arranged in groups which show their mode of origin from pre-existent parent-cells ; but each of these groups does not represent the whole product of one archesporial cell ; probably each of the latter gives rise to two or even three of the groups there clearly defined. I am not able to state definitely the number of archesporial cells in this plant, but it is probably about six. Below the sporogenous tissue is the formative tissue of tapetum and stalk, of which the cells have obviously undergone repeated divisions, so as to raise the sporogenous mass up from its originally deep-seated position ; the point marked (×) is the middle of the sausage-shaped sporangium, only half of which could be shown in the figure.

The general form of the sporangium as it approaches maturity, and its relation to the sporophyll and axis, will be seen from figs. 92 (*a, b, c*), and it is to be noted how closely the sporangium is surrounded by the sporophylls, and laterally by out-growing flanges of the axis, so that it is almost completely protected from evaporation from its surface, or from the direct sunlight, during its earlier period. The form of the sporangium is slightly curved (fig. *b*), and it is inserted by a short and moderately massive stalk close to the axil of the sporophyll.

Taking all the characters together, the strobilus of *Phylloglossum* is, both in its development and in its mature structure, strikingly similar to a simple strobilus of some species of *Lycopodium;* comparing the sporophyte of these plants as a whole, the most salient points of difference are first the size, and secondly *the absence from Phylloglossum of any representative of the leafy and often branched vegetative axis,* which as a development of considerable, often of great extent, precedes the production of strobili in *Lycopodium.*

Summary for Phylloglossum.

The most important results from this examination of *Phylloglossum* for purposes of our present comparison are these :—

1. The abrupt transition (with only few and inconstant intermediate steps) from the protocorm with its protophylls, to the strobilus with its sporophylls and sporangia.

2. The close similarity of the strobilus to that of *Lycopodium*.

3. The origin of the sporangium corresponds in detail to that of some species of *Lycopodium*, such as *L. Selago*.

4. The archesporium consists of about six cells, only one of which appears in each radial section.

LYCOPODIUM.

In the observations above described, and in those embodied in other works referred to, I see no material obstacle to the application of our working hypothesis to *Phylloglossum*, according to which the protocorm would be compared with the sterile seta of Bryophyta, of which it would be an elaborated example; the strobilus would also be an elaborated type of sporogonial head. It must, however, be remembered that the embryology of *Phylloglossum* is still unknown, while the sexual generation has never been seen; till observations on these are made, such a question cannot be considered as finally settled.

The relations of parts are by no means so obvious in the genus *Lycopodium*; in certain species, as already shown by TREUB, the protocorm is represented in embryonic stages (*L. inundatum, salakense, cernuum*), but in these it plays an unimportant part, as compared with the vegetative development which follows, and the question will arise how this more prominent vegetative phase of *Lycopodium* is to be regarded. The strobilus in many species is a clearly differentiated part of the plant (*e.g., L. clavatum*); in other species, however, there are peculiarly alternating sterile and fertile zones on the plant (*e.g., L. Selago*), and the question for us will be how are such characters to be harmonized with what has been seen in *Phylloglossum*, and how will they fall in with our working hypothesis? But before such questions can be discussed, the detailed description of observations which have been made on *Lycopodium* must be given.

The most detailed descriptions hitherto published of the development of the sporangium of *Lycopodium* are those given for *L. Selago* by GOEBEL,[*] and by SADEBECK[†] for *L. clavatum*. These descriptions are based chiefly upon radial sections through the strobilus, and though the appearance in tangential section is alluded to, no detailed description is given of the tissue so exposed; transverse sections appear not to have been examined by either writer. Obviously sections will be required in all three directions, radial, tangential, and transverse, in order to obtain a clear understanding of the structure and development of the sporangium; this is more especially needed where, as in this genus, the sporangium is large and complex in structure. SADEBECK remarks[‡] that the recognition of the details of the archesporium is a matter of no great moment; in dealing with plants, which are allowed to be the

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[*] 'Bot. Zeit.,' 1880, p. 561, &c.
[†] SCHENK's 'Handbuch,' vol. 1, p. 313
[‡] *Loc. cit.*, footnote, p. 318.

surviving relics of a flora with a great history in the past, and to show affinities to the Bryophyta, all details have their importance, more especially those which relate to the primary spore-producing members. GOEBEL appears to have examined only one species in detail, and to have assumed that the details are virtually the same for all; in so comprehensive a genus as *Lycopodium* it is desirable to examine and compare a number of species, of as divergent types as possible.

Accordingly a very large number of serial sections have been prepared from different species of the genus; the strobili have been cut radially, tangentially, and transversely, and upon these sections a comparative study of the development of the sporangia from their earliest stages has been based. The results will now be described in detail, and it will be shown that there is not only a considerable variety in the form of the sporangium in different species, but also in the mode of origin and number of the archesporial cells.

It may further be added that the sections were cut in series, by the rocking microtome: being extremely thin, there was no need of clearing by potash or other agents: as each section included only one layer of cells, or at most parts of two, there was no need of focussing down into the thickness of the section in order to obtain results, a method which has frequently been a source of error in developmental studies. Finally, the recognition of the archesporium in early phases of development has not been based merely upon the refractive power, or granular character of the cell-contents, but in cases of doubt the decision has been arrived at by comparison of other sections illustrating the course of development, rather than by recognition of differential characters in the individual specimen; for these differential characters often appear relatively late, and are, at best, an uncertain guide.

L. Selago. L. (figs. 34–49, and fig. 92, *d, e, f.*).

This species having been the subject of the most detailed previous study, will be first described. The sporangium originates on the upper surface of the sporophyll, and close to its base; it is, at the time of separation of the tetrads, a slightly curved body, of which the form as seen in radial, tangential, and transverse sections will be best recognized by reference to the figs. 92, *d, e, f.* It is specially to be noted that in the radial section the stalk is narrow in proportion to the size of the head of the sporangium, while, as seen in tangential section, the stalk measures rather less than one-third of the total width of the sporangium. The sporophyll covers in the young sporangium only partially; and, together with the next higher leaf and flanges of the stem below the insertion of the lateral leaves, forms a much less complete protection than is the case in some other species.

In examining radial sections through the sporangium those have always been selected which include the vascular bundle of the sporophyll; it is not sufficient in the case of a sporangium of tranversely elongated form, like that of *Lycopodium*, to ascertain that the section of the strobilus is strictly radial, for in such a section the

sporangium may be traversed obliquely ; if, however, the sporophyll be cut radially, so also must be the sporangium which it subtends. As seen thus in radial section the sporangium appears to originate from a single cell, but this is only one of a series which lie at the base of the sporophyll. This cell divides by anticlinal walls, so disposed as to cut off lateral parts from a larger cell which lies between them (× figs 34, 35); the lateral cells undergo subsequent subdivision, but apparently not according to a strict rule, as will be seen on comparison of figs. 35 to 41 ; the larger central cell (×) has a square base ; by periclinal walls there are cut from it a basal cell (iii) which after further subdivision contributes to the central part of the stalk ; and a superficial cell (i), which forms part of the wall of the sporangium : the cell (ii), which lies between these, is the *archesporium*, and it is thus unicellular, as seen in radial section. The description thus far coincides with that given by GOEBEL, and fig. 36 may be compared with his fig. 8 ; excepting some differences of position of the less important walls the drawings correspond ; it is not however to be expected that the correspondence should extend to minute details, in the case of relatively bulky masses of tissue such as these. I am unable to endorse the details of the subsequent steps as described by GOEBEL. He illustrates by his fig. 9 how, at a later stage, the sporangium consists of a wall of a single layer of cells, and three rows of cells enclosed by it. The archesporium is stated by him to be derived from the central more strongly-growing row, while lateral rows are curved to one side, and take no direct part in the formation of spores ; it is to be noted that GOEBEL gives no account of how these three rows of cells originate, and in accurately radial sections I have never seen such an arrangement. The structure of the sporangium, which I found to be typical though not constant at this later stage, is that shown in fig. 37 ; the cell (i) of fig. 36 has undergone anticlinal divisions, so that the wall, of which it forms a part, consists of a single line of cells ; cell (ii), which is the archesporium, divides longitudinally and transversely, so as to form a group of sporogenous cells, which abut on three sides directly upon the wall of the sporangium, while the base is in contact with the tissue derived from cell (iii) ; this has undergone longitudinal division into two, and these cells divide repeatedly in a transverse direction, so that two primitive rows of cells, not three, occupy the interior of the stalk. But this almost diagrammatic regularity may be often absent, and some sporangia present appearances such as those in figs. 38 and 39 ; the explanation of such irregularities as these is to be found in the fact that the archesporium consists not of one cell, but of a row of cells, and that the walls separating these do not always run in radial planes, but sometimes obliquely, so that even a perfectly radial section, cut very thin, may include parts derived from distinct archesporial cells ; this has probably been the case for the parts *a* and *b* of the figs. 38 and 39. In the latter the periclinal division of the cells of the wall has begun ; this takes place as GOEBEL has described it, the superficial cells dividing periclinally ; the inner of the resulting layers again divides, the innermost layer being the tapetum ; there is often some irregularity in the details, and it is to be specially

noted that the second divisions are delayed at the apex, where the line of dehiscence of the sporangium will be (*d*, fig. 40). The head of the sporangium meanwhile enlarges, the sporogenous mass of cells having grown, and having undergone repeated divisions the spore-mother-cells round themselves off, separate from one another, and are freely suspended in a fluid mass ; they then divide into tetrads in the manner already well known. The structure of the radial section of the sporangium at the time when all the essential parts are laid down, is thus shown in fig. 41 ; the wall consists typically of three layers, of which the outermost is the permanent wall ; irregularities occur, however, by which the number of layers is in part increased to four, or even more layers ; the stalk, originally consisting of three rows of cells, remains permanently narrow as compared with other species, and shows 5–7 rows of cells.

Since the sporangium, when mature, is a reniform body, tangential sections, which follow the plane of curvature, will give more certain results than transverse sections in determining the origin, number, and form of the archesporial cells. Hitherto, little is definitely known on this point. GOEBEL does not give any detailed account of the tangential section (*loc. cit.*, p. 564). Fig. 42 shows a tangential section, through a sporangium of nearly the same age as fig. 36, and the corresponding cells are similarly numbered. It thus appears that the number of archesporial cells (which are here shaded) is at least seven, but a comparison of other sporangia shows that the number may vary. Examination of their arrangement, and relations to one another, shows that they are not referable in origin to a single cell, though this was suggested by GOEBEL as not improbable. It seems rather that in this section they are referable to at least three, but a comparison of other sporangia has not disclosed any fixed number of archesporial cells, nor any definite mode of their origin. The sporangium, which appears at first (fig. 42) as a simple projection, soon begins to extend right and left (fig. 43), projecting beyond the limits of its stalk, and assuming ultimately the kidney-like form shown in fig. 92, *e* ; the archesporial cells meanwhile divide (figs. 43 and 44), in a manner which explains itself from the figures ; the cells of the wall also divide periclinally, and in accordance with what has been seen in radial sections. The tissue derived from the cells marked (iii.), and the adjoining cells, is worthy of attention : the cells grow and divide repeatedly, forming a massive pad below the sporogenous tissue, which apparently presses the latter outwards, especially at the central part, and the whole sporangium assumes the curved form. This pad of neutral tissue is smaller here than in several other species, but it is well to note its existence, and as it will be an important feature in the subsequent argument, it may be called the *sub-archesporial pad*.

It remains to describe the appearance of the sporangium in transverse section. This has been alluded to by GOEBEL (*loc. cit.*, p. 564), and the multicellular character of the archesporium was observed by him, but no drawings were published. The sporangium appears as an elongated and massive projection, and when viewed externally and from above, shows no clearly-defined series of cells which could be

distinguished as the cells (ii.) of figs. 36 or 42 ; the segmentations seem to be irregular, and this will coincide with the difference of details of segmentation as seen in other sections (fig. 45). The series of archesporial cells being curved, it is clear that a transverse section will cut only the middle cells of the series transversely, while those right and left from them will be cut obliquely ; this must be remembered in the interpretation of the sections. When cut transversely (fig. 46), the young sporangium shows the series of archesporial cells, of which six or more may be traversed. The wall of the sporangium consists at this stage of a single series of cells ; at the poles of the sporangium the appearance of a doubling of the walls may be presented, but if reference be made to fig. 42, and the plane of section be taken as the line *s, s*, it will then be understood how this apparent doubling comes about. The sporangium, in such sections as fig. 46, is adherent to the sporophyll. This is explained by reference to fig. 36, in which the line *s, s* will indicate the plane in which the sporangium of fig. 46 has been cut. The subsequent development is illustrated by figs. 47, 48, and 49, and, after the foregoing descriptions, these will call for no detailed explanation. It may be noted, however, that the wall of the sporangium on the side next the sporophyll is commonly thicker than on the adaxial side ; this may be seen early in the radial sections (figs. 37–39), and comes out clearly in the transverse sections also (figs. 48 and 49).

The origin of the tapetum is, as GOEBEL has described it, partly from the cells which form the wall of the sporangium, but partly also from the products of cells (iii.) in figs. 36 and 42. The question may be brought up whether the whole of the sporogenous mass be really derived from the cells recognized as the archesporium, or whether other cells may also contribute to the mass ; for instance, such cells as those marked (×) in figs. 46 and 47 might suggest the idea of such additions; other sections, somewhat irregularly cut, might also be thought to support this ; it has, however, been shown that the cells marked (×) owe their appearance to the oblique direction in which the plane of section traverses the lateral parts of the sporangium, and a similar consideration of the details in other cases suffices to show that in this species the sporogenous mass is referable in its origin entirely to the archesporium as above described.

It is well known how *L. Selago* bears on one and the same axis successive fertile and sterile zones ; the character of the leaves, however, varies but little, the sterile foliage leaves being in all essential points similar to the sporophylls. An examination of the leaves about the limits of these zones shows that in the axils of those apparently sterile, a more or less completely arrested sporangium is commonly to be found, so that the transition from the one to the other is a gradual one. The simple fact, so easily observed in this species, is found also to hold for others, and its importance will be brought out more prominently on a later page (p. 535).

Results from study of L. Selago. L.

The chief facts regarding the development of the sporangium in *L. Selago* may be summarised as follows :—

1. The sporangium originates at the base of the sporophyll, as a transversely extended cushion, consisting of many cells, arranged without strict regularity.

2. The archesporium consists of one row of hypodermal cells, six or more in number, which give rise to the whole of the sporogenous mass.

3. The tapetum is derived partly from the primitive wall of the sporangium (from the innermost of the three layers resulting from its division), partly from the cells which lie directly below the archesporium.

4. The pad of tissue below the archesporium grows into a convex mass, which projects so as to give a curved form to the sporogenous tissue; this is however less marked than in some other species.

5. The stalk of the sporangium is narrow, consisting at first of three rows of cells as seen in radial section; these subsequently increase by division to five, six, or seven.

6. The plant shows successive sterile and fertile zones, and there is no sharp limit between the fertile strobilus, and the sterile, or vegetative part; about their limits arrested sporangia are to be found in the axils of the leaves.

L. phlegmaria. L.

Certain other species of *Lycopodium* which have been examined show a near approach to *L. Selago* in the characters, both mature and developmental, of the sporangium; of those we may take, first *L. phlegmaria,* and it is somewhat remarkable that the similarity should exist in species which in habit and habitat are so different as these two species are. The general form of the sporangium and its relation to other parts of the strobilus are shown in figs. 92, *g, h, i* ; it will be noted that the similarity to *L. Selago* is most pronounced in the radial sections (figs. 92, *d, g*) ; in the tangential section the sporangium of *L. phlegmaria* is much more curved, and the stalk much narrower than in *L. Selago* (figs. 92, *e, h*) ; this character is well seen from the earliest stages of development (fig. 50), which also shows that the archesporium is referable in this species to not more than four, and probably even to two cells, while the stalk is also referable to the division of two cell-rows.

L. nummularifolium. BLUME.

There is an obvious similarity between the strobili of this species and of *L. phlegmaria.* Examination of sections of the sporangia shows close similarity of details, so that there is no call for a special description; it may, however, be

remarked, that occasionally extra periclinal divisions take place in the wall of the sporangium, thus increasing the mass of tissue composing it. Such divisions are occasionally seen in *L. Selago*, but are a characteristic feature in *L. dichotomum* (see below).

L. carinatum. DESV.

A form of the sporangium similar to that just described is found in *L. carinatum*, though in this species the actual size of the sporangium is much greater than in either of the preceding species. An examination of figs. 92, *n, o, p,* will show the resemblance to *L. phlegmaria*, both in the form of the sporangium, and the proportionally delicate stalk, and also in the lax manner in which the young sporangium is protected externally by the axis and sporophylls; all these peculiarities are thus shared by the four species which accordingly form a natural group as regards this character. In fig. 51 is shown a transverse section of the sporangium of *L. carinatum*, cut so as to traverse the sub-archesporial pad (*s.p.*) at a point immediately above the insertion of the stalk; this was drawn for purposes of comparison, and will be referred to later on. The shaded portions represent the sporogenous tissue. Specimens of this, and of the two preceding species, were kindly sent to me from Buitenzorg, by Dr. TREUB.

Lycopodium dichotomum. JACQ.   (= *L. mandiocceanum.* RADDI.)

Specimens of this species were supplied to me from the Botanic Garden of Brussels. These show the sporangium to be similar as regards form to those of *L. phlegmaria* and *carinatum* (fig. 92) though the stalk is somewhat more massive, and the sub-archesporial pad is more largely developed. The chief interest, however, in the sporangia of this species lies in the fact that the wall of the sporangium is of unusual thickness. A detailed examination shows that the wall of the sporangium consists of some 4-7 layers of cells (figs. 52, 53); the number is not exactly defined, nor is the arrangement of the cells very regular; the outermost layer develops with thicker walls, as in other species; the innermost is the tapetum, while between these intervene several layers of thin-walled cells, which evidently have been increased in number by periclinal divisions (fig. 52). The development shows that this is the case, and that the difference between this and other species is due to additional periclinal divisions in the wall, and not to the contribution of tissues from the sporogenous mass to the tissues of the wall.

The interest of this departure from the usual type of *Lycopodium* rests on comparison with other forms; it will be shown later, that in "Brown's Cone" of *Lepidostrobus* the wall of the sporangium is more bulky and complex in structure than in other *Lepidostrobi*, or in most species of *Lycopodium*; it is generally known, also, that the wall of the sporangium of *Ophioglossum* is rather similar in structure to this of *L. dichotomum*; at present it will suffice to note these facts, which will be dwelt upon more fully at a later page. But even in sporangia of *L. Selago* and

L. nummularifolium a tendency towards this more bulky development of the wall is to be seen, especially near the base of the sporogenous mass ; a comparison of figs. 40, 41, 49 of *L. Selago* shows that periclinal divisions have here and there increased the thickness of the wall to four layers in the lower part of the sporangium ; we have but to imagine such divisions to extend upwards and to be more numerous, and the more complex wall of the sporangium as in *L. dichotomum* would be the result.

In other respects the development and structure of the sporangia of this species do not present characters requiring special description.

L. inundatum. L.

We may pass on now to species in which the sporangium is, even from the initial steps, of a more bulky character than in the *Selago* type. *L. inundatum* may be taken as an intermediate example (figs. 92, *k*, *l*, *m*) ; here the stalk is shorter and more massive than that in the preceding species, while the form of the sporangium approaches more nearly to that of *Phylloglossum*. The details of protection of the sporangium while young by the other parts of the strobilus also resemble those of *Phylloglossum*, though the protection is even more complete than in that plant.

Turning to the early stages of development it is seen that the sporangium originates as a more massive structure, showing a gentler and wider curve as it arises from the sporophyll. The sporogenous tissue appears here to be derived not from a single archesporial cell as seen in radial section, but two at least take part in its formation (fig. 54, shaded cells), while their relations are such as to show that the periclinal divisions, by which they were separated from the superficial cells, were formed independently in the two adjoining cells. Traces of this mode of origin may be seen in much later stages, and fig. 55 shows how the sporogenous tissue may still be recognized as composed of two parts, which are referable to the two initial archesporial cells. These sporangia have not been followed out into further detail. It may be mentioned, however, that seven or more initials are to be seen in the tangential section of a young sporangium. When the development of the sporangium in *L. alpinum* and *L. clavatum* has been described it will be seen that *L. inundatum* occupies an intermediate position between the more massive type and that of *L. Selago*, both as regards the mature form and also in respect of the earlier stages of development of the sporangium. It will subsequently be shown also that this mode of origin of the sporangium in *L. inundatum* approaches more nearly to that in certain species of *Selaginella* than any others which have been examined.

At the base of the strobilus of this species, where the gradual transition takes place from the sterile foliage leaf to the sporophyll, completely arrested sporangia are often found in the axils of the leaves. Passing upwards from these to the typical part of the strobilus a gradual passage to the typical sporangium may be traced (see p. 535).

L. clavatum.   L.

The sporangium of this species is somewhat distinct from those which have already been described (figs. 92, *t–x*). Instead of the stalk being relatively thin, it is very short and massive in *L. clavatum*, while the sporogenous tissue forms a strongly-curved and rather less bulky zone, which fits immediately over the sub-archesporial pad ; this is here very massive, and, as seen in tangential section (fig. 92, *u*), projects far up into the sporangium : it has, moreover, this peculiarity, observed in no other species of *Lycopodium*, that its outline is distinctly convex in the radial section (fig. 92, *t*), and may even sometimes extend, as the sporangium becomes matured, into irregular processes, which project into the developing mass of spores ; in this we probably see a means of more ready transfer of nutritive materials from the base of the sporangium into the very large mass of spores. This character will be found of value when we come to compare it with the sporangia of *Lepidodendron*.

The sporangia are exceedingly well protected during their early phases. This may be gathered from figs. 92, *u, v, w*, in which it will be seen that the leaves closely invest the sporangia on all sides ; it is, moreover, to be remembered that these drawings are not made from the living specimen, but from such as have been treated with various hardening agents which would cause shrinkage ; it is probable that in the living state the chinks between these would be still smaller than as shown in the drawings. So close an investment is in broad contrast to the insufficient protection of the sporangia in *L. dichotomum, Selago*, and other species.

The sporangium of *L. clavatum* has been the subject of developmental study by SADEBECK (SCHENK's 'Handbuch,' vol. 1, p. 313), who has figured various phases of its growth as seen in radial section ; but tangential and transverse sections are not mentioned by him. In the interpretation of the radial sections he has obviously been influenced by GOEBEL's paper ('Bot. Zeit.' 1880) so often referred to in this memoir ; notwithstanding his working on a different species from GOEBEL, he still professes to a correspondence in results, so far that he refers the sporogenous tissue of *L. clavatum* to a single hypodermal cell as seen in radial section. This account is widely divergent from the results which I have obtained, and verified by comparison of radial, tangential, and transverse sections of sporangia in various stages of development. The difference lies in the recognition of the limits of the archesporial tissue ; now it is only by comparison of sections of sporangia of various ages cut in different directions that reliable views can be obtained on the recognition of the archesporium ; the mere distinctive qualities of the protoplasm and wall are not sufficiently defined in the earliest stages to make it possible to recognize the limits of the archesporium with certainty by their means. If, however, a comparison be made between his drawings and those which illustrate the description which I am about to give, it will be seen that SADEBECK's drawings of the cell-net were more reliable than his recognition of the archesporium.

The sporangium, in this species, arises first as a broad flat growth on the surface of the sporophyll. A radial section through one which has just begun to undergo the characteristic cell-divisions shows hardly the slightest convexity of the surface (fig. 56); the part where the sporangium is to be formed is occupied by regularly arranged superficial cells, rather deeper than they are wide; in these, periclinal divisions begin to appear, of which but one is seen in fig. 56; but in fig. 57 two such divisions are already complete, and in a slightly older sporangium, shown in fig. 58, there are three. This is, in fact, the common number of such divisions in the young sporangium as seen in radial section, and a comparison of a large number of sections, both at these early stages, and also older, leads clearly to the conclusion that the sporogenous tissue is not referable in its origin to one cell as seen in radial section, but usually to three. A careful comparison of figs. 56, 57, 58 clearly shows that the periclinal divisions appear independently in the three superficial cells. The outer cells thus defined form the wall of the sporangium; they divide first anticlinally, as the sporangium gradually enlarges (fig. 58), then also by periclinal walls (fig. 59), while the inner layer thus produced again divides periclinally (fig. 60); the result is as in *L. Selago*, &c., and the three resulting layers develop further in virtually the same way. The three inner cells (fig. 58), undergoing repeated divisions, together form the sporogenous mass (fig. 59, shaded), and it is often possible, even in radial sections of advanced stages of development, to distinguish these cell-groups, resulting from the division of the three parent cells. It is to be noted that these cell-groups are of unequal size, the smallest being usually that most remote from the stem (compare figs. 58, 59, 60). The sub-archesporial tissue is rather irregular in its further development; sometimes the limit between it and the sporogenous tissue is even and regular (fig. 59); it often becomes convex as maturity approaches (fig. 92, *t*), but it is not unfrequently very irregular, as is the case in fig. 60, where there are sharp angles in the line of limitation of the sporogenous tissue, these angles coinciding with the limits of the masses of tissue derived from the three parent cells. In some sporangia this tissue even grows upwards as projecting teeth, which, as the sporogenous cells separate from one another and become rounded off, force them aside, and proceed a considerable distance into the mass. This point is of interest for comparison with *Lepidostrobus* (see below, p. 526).

Returning to early phases again, abnormal cell-divisions are occasionally met with; these are not to be ascribed to oblique cutting of the sections, for the presence of a continuous vascular strand has in each case been taken as a criterion of the section being truly in the radial plane. In such sections it may occasionally be seen that the superficial cells divide again, very early, by periclinal walls; the inner cells thus formed (*o, o*, figs. 61, 62) are believed to be added to the sporogenous tissue, but this has not been distinctly proved. In view of the fact that such additions to the sporogenous tissue are constant in *Equisetum*, as above described, there can be no *à priori* objection to this view, which will also gain further strength from facts to be described in the case of *Isoetes*.

Neither GOEBEL nor SADEBECK gives details of tangential sections; both leave the question open as to the number of cells of which the archesporium is composed, or, indeed, whether all may not be referable to a single parent. SADEBECK (note, *loc. cit.*, p. 313) remarks that this question is unimportant; to this view I cannot agree.

Tangential sections of sporophylls of about the same age as that shown in fig. 56 solve the question at once (fig. 63); here the same regularly disposed superficial cells are found, as have been noted in the radial sections; some of these have already divided by periclinal walls, others are still undivided. It is plain from such a case as this that a large number of parent cells divide thus to form the internal archesporium and external cells of the wall, and in fig. 63 the number appears to be at least twelve. In fig. 64 the archesporium is clearly defined, while the cells of it have already undergone further divisions, and the whole sporogenous mass is beginning to assume the characteristic form already noted. The cells of the wall have not yet begun to divide in a periclinal manner, but in fig. 65 a still more advanced stage is shown, and a comparison of this with fig. 60 will be found to agree in essential points, though the latter was taken from a slightly older sporangium.

The tangential sections show from the first that the lower limit of the archesporium is not a regular line; as the sporogenous tissue grows older the limit becomes more irregular, owing to the frequent growth upwards of the sub-archesporial tissue, which extends as more or less projecting processes between the sporogenous cells; these processes are irregular both in occurrence and form. Fig. 66 shows one of these in a sporangium in which the spore-mother-cells have not yet separated, or divided into tetrads; while in fig. 67 a later stage is seen, after the tetrad division; the cells of the processes are thus beginning to be disorganized, their function as purveyors of nourishment being almost complete. These figures will supply material for interesting comparisons with similar developments in certain specimens of *Lepidostrobus* and in *Isoetes* (see p. 526, 532).

Turning now to transverse sections, we shall seek in them for some corroboration of the results acquired from study of the radial and tangential. It is to be remembered, however, that the sporangium is a curved body, and, accordingly, that if the central part of the sporangium be cut in a tangential plane, the parts right and left of it will necessarily be cut obliquely.

That the sporangium is a broad and massive structure is borne out by a superficial section, such as that shown in fig. 68, which exhibits the external cell-net. A section of a similar sporangium at a lower level will traverse the sporogenous tissue, as in fig. 69, and if we imagine this cut in a plane (x, x), the result would be nearly similar to that shown in fig. 58, the heavier lines in both cases indicating the limits of the original archesporial cells. Again, at a later stage, the sporogenous tissue is shown in fig. 70; the drawing here stops short at the median plane, and it is not difficult to see how this will coincide in all essential points with what is seen in radial section in fig. 59. It will be unnecessary to pursue these comparisons further

into detail, the figures should sufficiently explain themselves to those who are accustomed to such comparisons.

The strobilus of *L. clavatum* is clearly defined from the vegetative region; it is borne on a long, upright stalk, which is covered by small, closely-appressed leaves. If sections of the base of the strobilus be examined, imperfect sporangia may frequently be found attached to the lower leaves, and corresponding in position, though not in ultimate development, to normal sporangia. Similar imperfect sporangia also occur at the upper limit of the strobilus, while the uppermost leaves are completely sterile.

Comparison of details of sporangial development in L. clavatum, and other species, especially L. Selago.

1. The sporangium is similar in position and in general form to that of *L. Selago*, but its body is more strongly curved.

2. The archesporium here consists of *three rows of cells*, each row being composed of a large number (about 12) of cells; thus the extent of the archesporium is much greater than in *L. Selago*: occasional additions to it seem to be made by cells cut off periclinally from the superficial cell at an early stage.

3. The tapetum is similar in origin to that in *L. Selago*.

4. The sub-archesporial pad is much more developed, and is at times extended as processes of tissue which penetrate the sporogenous mass for a short distance.

5. The stalk of the sporangium is much thicker and shorter than in *L. Selago*.

6. Arrested sporangia are frequently present, and may be found either at the base or the apex of the strobilus.

6. *L. inundatum* may be looked upon as an intermediate link between the type of sporangium of *L. Selago* and that of *L. clavatum*, both as regards form of the sporangium and complexity of the archesporium.

L. alpinum. L.

This species shows great similarity to *L. clavatum*, both as regards the form of the sporangium and the very complete protection of it while young by the adjoining sporophylls (figs. 92, *q*, *r*, *s*); but the stalk of the sporangium is not quite so short, nor is the sporogenous part so strongly curved, while the sub-archesporial pad does not project so convexly, as seen in the radial section (fig. *q*).

The sporangium here also originates as a very broad outgrowth, extending from the first over numerous cells of the radial section (figs. 71 and 72). At least three cells in each radial section are involved in the origin of the archesporium (figs. 73, 74), but the position of the successive divisions does not appear to be strictly fixed, so that it is difficult to recognize the limits of the archesporium in very early stages such as

those in figs. 71 and 72.   Occasionally here also further periclinal divisions appear in the superficial cells, by which subsequent additions may be made in the archesporial tissues, as in *Equisetum*.   The cells marked (*x*) in fig. 74 are believed to be thus added on to the archesporium ; this is, however, unusual.

The further development proceeds in essential points as in *L. clavatum*, and it would hardly have been necessary to illustrate it by fig. 75, had it not been that the vascular bundle showed, in the one case figured, a slight extension upwards toward the sporangium.   I do not wish to make more of this than the facts warrant, but the drawing is a faithful representation of what was seen in this exceptional case.   It is a matter for remark that these sporangia of *Lycopodium*, though they are of so considerable a size, have no vascular supply ; in the whole genus this one case is the only trace I have seen of any extension of the vascular system in the direction of the sporangium.

Tangential sections show that the number of archesporial cells is large ; and fig. 76, which is at a phase of development intermediate between those of figs. 63 and 64 of *L. clavatum*, shows very beautifully that the number of these is twelve.   The limit of the sub-archesporial pad often becomes irregular as the development proceeds ; even in an early condition (fig. 76) it is far from being a straight line, but it becomes more irregular with age (fig. 77), the cells of this sterile tissue forcing their way upwards into the sporogenous tissue, and occasionally forming multicellular processes.   The development of it, however, is not carried to so great an extent as has been already noted in *L. clavatum*.

Beyond the facts now described, the structure of the sporangium of *L. alpinum* calls for no further remark ; it is obviously similar, in its main aspects, to that of *L. clavatum*, notwithstanding the rather marked difference of external appearance of the two species.

Abortive sporangia are commonly found in this species, both at the upper and lower limits of the strobilus ; they show, perhaps better than in any other species, the gradual steps of transition from the fully mature to the completely abortive condition.

SELAGINELLA.

In the species of *Selaginella* which have been examined similar abortive sporangia are found at the base of the strobilus ; the larger the number of examples of this that are disclosed, the more important does it become that some adequate explanation of the phenomenon should be given.   (See below, p. 535.)

Turning to the development of the normal sporangia in *Selaginella*, the most exact account hitherto given is that by GOEBEL (' Bot. Zeit.,' 1881, p. 697, &c.): he remarks on the difficulty of following the details of segmentation, owing to the small size of the cells ; this may account for the divergence of my results from those of GOEBEL, and it should make one cautious in making definite statements on minute details.

As regards the position of the sporangium of *Selaginella*, I am able to endorse Goebel's statement (*loc. cit.*, p. 697) as to the position of the sporangium in *S. spinosa*, P. B. (= *S. spinulosa*, A. Br. = *S. selaginoides*, Link): it originates from a group of cells of the axis which lie at the axil of the sporophyll (figs. 80–86), and immediately above those which give rise to the leaf itself. In *S. Martensii*, however (fig. 78), I find the sporangium to originate on the axis, distinctly above the sporophyll. The mere fact that there is variety within the genus in this much discussed and greatly overrated character, should show sufficiently that, however interesting its morphological bearings may be, it is not a point of much systematic importance.

The sporangium of *S. spinosa* is eusporangiate, arising from a number of cells, as seen in radial section; it is possible, however, that these may all be ultimately referable in origin to a single cell (compare figs. 80, 81, 82). The young sporangium may thus be recognized in the radial section as consisting of three cells, which early undergo periclinal divisions to form three cell-rows (figs. 80, 82); these may occasionally show anticlinal divisions also (figs. 81, 83). Goebel now describes how the middle row of cells grows more strongly than the peripheral ones, while the hypodermal cell of this series forms the archesporium;[*] thus, according to his description, the sporogenous tissue in any radial section is referable to the subdivision of a single cell. I do not deny that this may sometimes be the case, but I have not been able to prove to my own satisfaction that it ever is so.

From many specimens which I have seen I find little evidence of the early preponderating growth of the middle row; it appears rather that the three rows grow about equally, while *sporogenous tissue originates from at least two of the rows of cells* above noted; for instance, in fig. 83, a continuous wall (x, x) divides the sporangium into almost equal halves; it is believed that both the cells shaded, though derived from different cell-rows, are archesporial cells; a comparison of drawings of sporangia in older states shows that a continuous wall, occupying an almost median position in the sporangium, is not uncommon (figs. 84, 87, walls marked x), and it is believed to correspond to the wall similarly marked in the younger sporangia (figs. 80, 82). If this be so, then, in figs. 83 to 87, the shaded mass of cells must have been derived from at least *two* of the rows of cells of the younger sporangium.

For confirmation of this result radial sections were also cut from *S. Martensii*, which species illustrates this point very clearly. The sporangium originates here from the axis, distinctly above the subtending leaf (fig. 78), as an outgrowth of at least two cells as seen in radial section; the wall dividing them occupies from the first a median position, and divides the sporangium into two equal halves; in this case a comparison of figs. 78 and 79 can leave little doubt that two cell-rows are involved in the formation of the archesporium.

It may be noted at once that a similar state of things is seen in *Lycopodium*

* *Loc. cit.*, p. 698, and Schenk's 'Handbuch,' 111; p. 388.

inundatum (compare figs. 54, 55), and that, as regards the origin of the sporangium, *Selaginella* seems to correspond more nearly to that species than to any other *Lycopodium* which has been examined.

Tangential sections of sporangia corresponding in age to those shown in figs. 84–86, demonstrate a similar fan-like tracery to that seen in the sporangia of *Lycopodium*. I have not been able to define exactly the number of primary archesporial cells, owing to the great difficulty of obtaining exact tangential sections of very young sporangia I believe, however, that the number is not less than three or four (fig. 88).

The further differentiation of the sporogenous tissue and of the tapetum may now be discussed. GOEBEL states (*loc. cit.*, p. 698) that the part of the tapetum which adjoins the outer wall is separated off from the archesporium, as distinguished from that of *Lycopodium*, which is derived from the cells of the wall. Though certainly a large part of the tapetum is so derived, I am not prepared to admit that this is its exclusive source, and find myself unable to endorse the account of the details as given by GOEBEL. Taking first *S. Martensii*, it will be noted that the two superficial cells of the sporangium in fig. 78 are relatively deep ; in fig. 79, which represents an older sporangium, they are relatively shallow, the cells immediately below them (*i, i*) would, according to GOEBEL'S description, be derived by division from the internal, not from the external cells, but the position of the walls, together with the less depth of the superficial cells in the older specimen, seem to indicate that they originate from division of the superficial cells. I venture to think that GOEBEL'S own fig. 14 ('Bot. Zeit.,' 1881, Plate 6) is quite open to the same interpretation, and that the cell marked (*t*) is the result of division from the next outer, and not from the next inner cell. An examination of numerous sections of sporangia of *S. spinosa* also strengthens the view that, at times, early periclinal division of the superficial cells may contribute to the internal tissue of the sporangium, though I have not found this point so clearly demonstrated in this species as in *S. Martensii*. My conclusion is, that the first periclinal division does not constantly define the archesporium, and thus separate the internal mass of the sporangium from the sporangial wall, but that, as observed with constancy in *Equisetum*, and occasionally in other plants (*L. clavatum* and *alpinum*), by periclinal divisions of the superficial cells additions may be subsequently made to the archesporium, but these additions are not constant.

Whatever may be the actual facts on the above point, there can be no doubt that after the archesporium has grown and undergone further segmentation, periclinal divisions take place in the peripheral cells of the resulting mass of tissue, these divisions separate off the tapetum from the sporogenous cells (figs. 87, 88, 89). Thus, as GOEBEL has already pointed out (*loc. cit.*, p. 698) the tapetum of *Selaginella* differs in its mode of origin from that of *Lycopodium*, but when we look back to the earlier phases, and recognize the periclinal divisions discussed in the preceding paragraph, the distinction of the two types appears to be not so deep a one as he described.

The further facts of differentiation of the sporangia, as mega- and micro-sporangia, are well known ; but there is, I believe, no published figure of the early stage of differentiation of the megaspore-mother-cell from the rest ; this is shown in fig. 91, as having undergone tetrad-division, and beginning to enlarge, while the other cells of the sporogenous mass remain undivided, lose their highly refractive contents, and become disorganized. It is hardly necessary to remark that here is a further example of a partial sterilization of the potential sporogenous tissue, and the tapetum of *Selaginella* is also another, since, though it is derived by segmentation from the sporogenous mass, its cells take no direct part in the formation of spores.

Summary of Results from Selaginella.

1. The sporangium is eusporangiate, and arises from the tissue of the axis, above the subtending leaf ; the position varies in different species.

2. The origin of the sporangium is similar to that of *Lycopodium,* and especially resembles *L. inundatum,* to which species the mature sporangium also is similar in form.

3. Two primary archesporial cells are usually present in each radial section, and these are derived, as in *L. inundatum,* from segmentation of two distinct cell-rows ; as seen in tangential section, the archesporium is referable to three or four such cell-rows.

4. The first periclinal divisions in these cell-rows do not always define the archesporium finally ; subsequent periclinal divisions may result in addition to the central mass, as has been proved for *Equisetum ;* but here the addition is less regular.

5. The tapetum results from tangential division of the outermost cells of the central mass ; the greater part of it originates as described by GOEBEL.

6. The tapetum is thus a sterilized part of the potential sporogenous tissue ; a further example of sterilization is seen in the megasporangium, where all the sporogenous cells are disorganized, excepting the one mother-cell of the megaspores.

7. Abortive sporangia are to be found at the base of the strobilus as in many species of *Lycopodium.*

LEPIDODENDRON.

The general characters of the sporangium in *Lepidodendron* are well known by the observations of R. BROWN, Sir JOSEPH HOOKER, WILLIAMSON, and others ; a concise statement of their results will be found in SOLMS-LAUBACH'S 'Fossil Botany' (Engl. Ed., p. 232, &c.). It is to be noted, however, that the comparison with the sporangia of living forms has been limited, partly perhaps owing to the incomplete knowledge of the sporangia of modern Lycopods. The facts which have been collected and described in the preceding pages make it possible to draw the comparisons closer.

while observation will, at the same time, be more carefully directed to points of detail which have hitherto received but slight attention.

The magnificent silicified cones in the British Museum have supplied the most important material. I take this opportunity of thanking the Keeper of the Botanical Department, not only for giving the free use of the specimens, but for readily agreeing to my suggestions for cutting new sections of parts of these rare fossils.

Lepidostrobus Brownii, SCHPR.

The large specimen of this species purchased for the British Museum in 1843, is the best preserved which I have seen, probably the best that is known to science. It is possible in microscopic sections of the cone to study its parts with the same detail as those of a modern Lycopod, while, since the sections in the British Museum are cut in all three directions, transverse, radial, and tangential, a very satisfactory knowledge of the structure both of the axis and sporophylls, and also of the sporangia, may be acquired. The radial and transverse sections were made for R. BROWN, and his account of them will be found in the Linnean Transactions (vol. 20, p. 469, Plates 23 and 24). It is remarkable how little attention has been paid to this memoir, and to the fossil to which it relates; even SOLMS-LAUBACH dismisses it with few words, and with the quotation of two figures which do it but scant justice ('Fossil Botany,' Engl. Ed., p. 238, and figs. 25A and 25B).

The structure of the axis is not our subject here; I have given a description of certain details of it elsewhere ('Annals of Botany,' 1893); we are more nearly concerned with the sporophylls and sporangia. These are of great size; each sporangium, when mature, being over half an inch in length, more than three-sixteenths in width, and almost of equal depth. The sporangia are so placed that the longer axis runs in a radial direction; each is closely applied to the upper surface of the sporophyll throughout the greater part of its length, while the basal portion of the sporophyll is elongated so as to accommodate the large sporangium (compare R. BROWN'S Pl. 23, B, C, Pl. 24, B). I have not been able to observe any structure comparable to the ligule in this fossil.

The sporangia are filled with very numerous microspores, which were described and figured by R. BROWN. Turning to the wall of the sporangium, R. BROWN remarks (p. 471), that it "appears to be double; the outer layer being densely cellular and -opaque, the inner less dense, of a lighter colour, and formed of cells but slightly elongated." A detailed examination of the sections shows that the wall consists of several layers of cells (fig. 93), of which the outermost consists of closely disposed prismatic cells, the walls of which were apparently much thickened; within this is a broad band of cells, consisting of four or more layers, with thin walls, and irregular, but compressed form; this wall is lined internally by an ill-defined band, which possibly represents the remains of the tapetum, and it directly adjoins the spores.

The interest of this rather complex structure depends upon comparison; among the various species of *Lycopodium* which have been examined, there are usually three layers of the wall, of which the innermost is the tapetum; but *L. dichotomum* is an exception, and it has been shown that in this species the wall is constructed in a manner very like that now described for *Lepidostrobus Brownii*, though the details of the outer layer are rather different. Again, a similar structure of the sporangial wall is found in the *Ophioglossaceæ*, a point noted by R. BROWN (*loc. cit.*, p. 471).

One of the most remarkable features in these sporangia is the existence of irregular processes which spring upwards from the floor of the sporangium, where it adjoins the sporophyll, and project a considerable distance into the cavity: they were noted *loc. cit.*, p. 471), and figured (Plate 24, fig. B), by R. BROWN, but appear to have entirely escaped the notice of subsequent writers. They are not scattered indiscriminately over the floor of the sporangium, but arise from a projecting ridge, which lies immediately above the single vascular bundle of the sporophyll, and follows its course almost the whole length of the sporangium; in transverse sections this ridge may be seen (fig. 94), but it is in tangential sections that it will be best recognized (figs. 95, 96), together with the processes of tissue which arise from it and radiate upwards into the cavity: it is obvious that the ridge corresponds to the mass of tissue which has been styled the "sub-archesporial pad" in describing the sporangia of *Lycopodium*. These process which thus arise from the sub-archesporial pad are irregular in outline and position; they consist of a parenchymatous tissue, which is directly continuous with the base of the sporangium (figs. 97, 98); the cellular structure, however, cannot always be recognized in the ultimate endings of the process, where they appear to have undergone considerable disorganization.

The question now presents itself, What is the real nature of these processes? Are they comparable to the trabeculæ of *Isoetes*, which are the result of partial sterilization of a potential archesporium, or are they merely outgrowths from the subarchesporial pad, such as those already described in certain species of *Lycopodium?* (compare *L. clavatum*, figs. 66 and 67; and *L. alpinum*, fig. 77). This question can only be decisively answered by observations of development, which can hardly be expected in fossils; there are, however, facts bearing on the point which may be acquired from the apical parts of this remarkable cone; it will be seen that in fig. 95, as the apex is approached, the sporangia are successively smaller; those close to the apex have been arrested in their growth, and appear to contain no spores; the cavity of the sporangium is, however, traversed by cellular processes, rising from the base, and extending upwards (figs. 99, 100); apparently these bands of sterile tissue extended to the upper wall of the sporangium, but I have been unable to establish beyond doubt the fact of a tissue-connection between them and the wall; had that been shown to exist, the correspondence between these and the sterile trabeculæ of *Isoetes* would have been demonstrated, and, from the appearance of some of the sections, I am inclined to the belief that this is their real nature; in the absence of such proof it

may be held as a possible alternative view, that they are merely upgrowths of the sub-archesporial pad, like those in certain species of *Lycopodium*, but on a larger scale, and that those upgrowths are specially large in the abortive sporangia; but I think the former view the more probable.

The second specimen of *L. Brownii*, from SCHIMPER'S collection, and now in the British Museum, has been figured by SCHIMPER ('Traité,' Plate LXII., figs. 13, 14). I have to thank the Keeper of the Botanical Department of the Natural History Museum for having this classical fossil cut in tangential section; the result is to demonstrate the presence of sterile trabeculæ similar to those seen in BROWN'S cone (fig. 102). From the enlarged subarchesporial pad processes of sterile tissue may be seen to arise, and project far into the sporogenous mass; but no evidence is to be found of the continuation of the trabeculæ outwards to the sporangial wall; it is, however, to be noted in this connection that the cone is not so well preserved as BROWN'S cone, and it does not include the apex.

It will be obvious, on comparison of RENAULT'S figures of *L. rouvillei* ('Cours de Bot. Foss.,' II., Plate 7, fig. 11), that M. RENAULT has observed in that species characters similar to those above described.

The presence of these sterile masses, whether they be true trabeculæ, or merely upgrowths of the subarchesporial pad, has its physiological interest. The sporangium is an unusually large one, and the spore-producing mass very bulky; the difficulties of supply of nourishment to so large a mass are obvious, and would be greatly diminished by processes of sterile tissue, such as these, extending far into the mass. It is further to be noted that they are inserted near to the vascular bundle of the sporophyll, and radiate from it; there can be little doubt that their function is the ready conveyance of nutrition to the developing mass of spores. Whether we can regard them as also performing a mechanical function, in supporting the roof of the sporangium in early stages, depends upon the question of the tissue connection, which I have been unable to decide.

Lastly, a formal comparison may be drawn between the sporangium of *Lycopodium* and that of *Lepidodendron*; at first sight the correspondence does not seem a close one. Comparing the tangential sections, it will be seen that fig. 96 of *Lepidodendron* is not unlike fig. 92B of *L. alpinum*, as regards the form of the sporangium, its relation to the sporophyll, and the subarchesporial pad, with its irregular upward projections. The radial and transverse sections, however, differ greatly, for the sporangium of *Lepidodendron* is extended radially, while that of *Lycopodium* is radially compressed; but, after all, this is only a difference on a larger scale, though similar in kind to that already observed between the different living species of *Lycopodium*; I have shown that in some species (*e.g.*, *L. Selago*) the archesporium is represented in radial section by a single cell, while in others (*e.g.*, *L. alpinum* or *L. clavatum*) it is represented usually by three. Why should the limit be three? Why not thirty-three?

Probably some such extravagant extension of the archesporium in a radial direction

existed in *Lepidodendron*, and its sporangium appears to be of the ordinary Lycopo-dinous type, but extended greatly in a radial direction, the result being a much greater possible production of spores, accompanied by risks for their proper nourishment, while there are special developments to meet those risks, and to provide for the nutrition of the developing spores.

Other Lepidodendra.

It has long been recognized that all *Lepidostrobi* do not correspond in the details of their sporangia; there is a type distinct from *L. Brownii*, which has already been figured and described by WILLIAMSON (Memoir III., 'Phil. Trans.,' vol. 162, 1872, p. 28, Plate 12, figs. 24, 25). This type appears also to be that which chiefly engaged the attention of Sir J. HOOKER in his memoir in the publications of the Geological Survey of Great Britain, vol. 11, p. 440. Sporophylls with their sporangia similar to those figured by these authors are shown as cut in tangential section in fig. 101, which is photographed from a specimen of my own, from Hough Hill, Stalybridge, supplied by Mr. Lomax. I find the details to correspond to Professor WILLIAMSON's description (p. 295); the wall of the sporangium consists for the most part of only a single layer of hard, prismatic cells, and is, thus, simple in structure as compared with the relatively thick wall of *L. Brownii*. The sub-archesporial pad projects only very slightly into the cavity of the sporangium, but from it arises, usually in a median position, a dark line, readily seen in the photograph, and represented in WILLIAMSON's figs. 23, 24, 25. Sometimes there is evidence of its bifurcating at the middle of the sporangium, while in other cases it appears of less regular form and position. Minute examination of the upper part of it does not disclose any definite cell-structure. Comparison with the mature microsporangia of *Isoetes* makes it almost certain that it represents a process of sterile tissue, for in the mature microsporangia of that plant the trabeculæ, which are of cellular construction, become so shrivelled that the individual cells are unrecognizable, the result being closely similar to what is seen in sporangia of *Lepidostrobus*; moreover, an examination of the base of the dark processes in *Lepidostrobus* shows, at times, evidence of distinct cells (fig. 102). These processes thus appear to correspond essentially to those already described in *L. Brownii*, though they are less numerous as seen in tangential section. In a transverse section of the strobilus, thus traversing the whole length of the sporangium, the brown line appears sometimes as a single continuous plate (compare WILLIAMSON's Plate 44, fig. 23), occupying the median plane in each sporangium. WILLIAMSON describes each as being "coextensive with the entire length of the sporangium." In a transverse section of *Lepidostrobus*, from Hough Hill, Stalybridge, supplied to me by Mr. LOMAX, I find the median plate very much as described by WILLIAMSON, though not extending the whole length of the sporangium (fig. 132). In another transverse section of *Lepidostrobus*, also supplied to me by Mr. LOMAX, from Dulesgate, I find two such brown lines, which run almost parallel for a considerable distance,

while a third less continuous line is seen to run for a short distance in a position between them (fig. 133). Thus we see that in different specimens of *Lepidodendron* these processes are of variable form, being peg-like upgrowths, or trabeculæ in *L. Brownii*, while in those last described they take the form of continuous plates. The resemblance of these plates to partial septa cannot be overlooked, and this inconstancy of character is a fact which will have its bearing on our general argument.

The question whether these processes are to be looked upon as sub-archesporial in origin, or as the result of a partial sterilization of the archesporium itself, must here also remain uncertain, in default of developmental data, which are necessary for deciding such a question. I think, however, that comparisons, on the one hand with *L. Brownii*, and on the other with *Isoetes*, justify the conclusion that in these simpler sporangia of *Lepidodendron*, also, the brown lines represent sterile tissue, which in the course of development of the spores has become disorganized, and the cells shrivelled out of shape.

Summary of Results from Lepidodendron.

1. The general arrangement of parts of the strobilus of *Lepidodendron* corresponds to that of *Lycopodium*.

2. The sporangium is greatly extended in a radial direction, and is to be looked upon as an extreme case of that radial widening of the sporangium which is seen in much less degree in *L. clavatum* or *L. alpinum*.

3. There are two types of sporangia of *Lepidodendron* : (*a*) that of *L. Brownii* in which the sporangial wall is several layers of cells in thickness, and the cavity traversed by rod-like masses of sterile tissue (trabeculæ); (*b*) those in which the wall consists of a single layer when mature, and the cavity traversed by one or more irregular plates of sterile tissue.

4. These sterile trabeculæ, or plates, arise from the sub-archesporial pad, and from early states of development seen in *L. Brownii* it seems probable that they are similar in their origin to the trabeculæ of *Isoetes*, but this has not been proved.

5. The physiological importance of these sterile processes projecting into the cavity of the sporangium is probably to forward supplies of nourishment more readily from the vascular bundle, above which they spring, to the mass of developing spores; they may also have served a mechanical purpose.

Isoetes Lacustris, L.

The genus *Isoetes* presents points of special interest in connection with the investigation upon which we are engaged. Though the development of the sporangia has been most carefully and successfully wrought out by Professor Goebel ('Bot. Zeit.,' 1880, p. 564), still there are certain details to be added, which are the outcome of my work in verification of his results.

Before describing these, a few preliminary remarks will not be out of place. The question of the systematic position of *Isoetes* has recently been re-opened by Professor VINES ('Annals of Botany,' vol. 2, p. 117), and on various comparative grounds he holds that *Isoetes* should be included in the Filicineæ, having more special affinities with the Eusporangiate Ferns. He still admits, however, that "there is some affinity between *Isoetes* and the Lycopodinæ" (*loc. cit.*, p. 123). I think that the comparison with *Lepidodendron*, rather than with modern Lycopods, greatly strengthens the affinity with the Lycopodinæ, more especially the comparison of their sporangia. I have already stated in the introductory pages of this memoir that I attach greater weight to the characters of the sporangium than to those of other parts of the sporophyte, and accordingly I am disposed to recognize a nearer affinity of *Isoetes* to the Lycopodinæ than Professor VINES would do. The description of details now to be given will, I think, justify this view.

The sporangium of *Isoetes* obviously corresponds in position to that of *Lycopodium*, though it differs from it in form. It arises from the upper surface of the sporophyll, at a point between the ligule and the base of the leaf. GOEBEL ascribes the sporangium to a group of cells, which extend, and divide by periclinal walls, and he continues, that according to his observations on *Isoetes lacustris*, it is usually the three uppermost layers of cells which give rise to the sporangium. For purposes of comparison with the Lycopodinæ, it is desirable to trace the origin of the sporangium back to earlier phases than that where it consists of three layers of cells. The stage shown in fig. 104 will serve as our starting point; the superficial series of cells lying at the upper surface of the leaf, between the ligule (*l*), and the base, here represent the parent cells of the sporangial wall and of the archesporium; the cell (V.), which gives rise subsequently to the "velum," must, however, be excepted. These cells divide by periclinal walls, and also anticlinally, to form two layers; the outer contributes the wall of the sporangium, the inner is the archesporium (fig. 105, shaded). As in the case of other sporangia, so here also the question arises whether the first periclinal division of the superficial cells clearly and finally divides the wall from the archesporium, or whether further periclinal divisions in the former contribute additions to the latter. We have already seen that such additions are made with constancy in *Equisetum*, while occasional and irregular periclinal divisions appear also in young sporangia of *Lycopodium* and *Selaginella*. GOEBEL has noticed such divisions occurring in the sporangium of *Isoetes* (SCHENK's 'Handbuch,' 3, p. 92), and remarks that this doubling of the layer of the wall is very common, though not so regular as in *Lycopodium*; evidently he regards it merely as a division of the wall of the sporangium into two layers. I also find periclinal division of superficial cells, after the first formation of the archesporium, to be not uncommon; it is to be noted, however, that such divisions occur *at very early stages* in the development of the sporangium; their common occurrence then (figs. 106-108), and the much less common appearance of the doubling of the superficial layer of the wall at older stages (such as

that shown in fig. 109), make it seem probable that the inner cells thus produced are contributed to the sporogenous mass. It is difficult to bring forward definite proof on this point, since the occurrence of such divisions is less regular than in *Equisetum*. A comparison of figs. 106–108, however, makes it appear extremely probable that the cells marked (×) in them are actually such additions to the sporogenous tissue, proceeding from a second periclinal division of certain of the superficial cells.

Be this as it may, the result of the segmentation is normally the formation of a superficial layer of cells forming the wall, while the subjacent cells, at first a simple row, become again divided by both periclinal and anticlinal walls, so as to constitute a continuous band several layers of cells in thickness (compare fig. 105 with figs. 106–108, and again with fig. 109). Below the archesporium a mass of tissue is found, intervening between it and the vascular bundle; as the sporangium grows this increases greatly in bulk, and forms the subarchesporial pad. It is to be noted that the sporangium does not extend the whole distance from the ligule to the base of the frond; the cell marked V (fig. 104) develops into the velum which intervenes between the ligule and the sporangium; also towards the base of the sporangium, a greater or less interval of sterile tissue is present from the first (figs. 105–109).

A comparison may now be drawn between the earliest stage of the sporangium of *Isoetes* (fig. 104) and that of *Lycopodium*; in certain species of the latter it has been shown that the sporangium is referable in its origin to a single cell as seen in radial section, and that a single archesporial cell is at a rather later stage disclosed in such sections (*L. Selago*, &c.). In another species (*L. inundatum*) the sporogenous tissue is referable to two cells as seen in radial section, while in others (*L. clavatum* and *L. alpinum*) the radial section traverses three original parent cells, all of which contribute to the sporogenous tissue. In *Isoetes lacustris* the whole plant may be regarded as a strobilus; most of its leaves are sporophylls; when one of these is cut in radial section the position of the sporangium relatively to it is very similar to that of the corresponding parts in *Lycopodium*, but a larger number of ultimate parent cells take part in forming the sporangium, the number traversed by the radial section being about four or five; this difference is, however, in accordance with the hemispherical form of the mature sporangium. The result of the earlier segmentation is, as in *Lycopodium*, the formation of a continuous sporogenous mass, protected by a simple outer wall. A comparison of figs. 104–106 of *Isoetes* with figs. 56–58 of *L. clavatum*, and with figs. 72–74 of *L. alpinum*, will show how close is the similarity of first origin of the sporangia in these plants.

GOEBEL has already traced the differentiation of the *potential archesporium* of *Isoetes* to form, on the one hand the sterile trabeculæ and the tapetum, and, on the other, the megaspores or microspores. I may be excused for dwelling again upon this point since it provides a most important link in the chain of my argument. In fig. 109 the potential archesporium of a microsporangium is shown, of very considerable extent, but still undifferentiated; it enlarges further, and the cells show further

divisions, the tissue meanwhile undergoing the differentiation described by GOEBEL ('Bot. Zeit.,' 1880, p. 565) to form the sterile trabeculæ (*tr.tr.*, fig. 110), and the fertile sporogenous masses (*sp.*, fig. 110); a layer of cells adjoining the wall of the sporangium is meanwhile divided off, and may be recognized as the tapetum (*t.*, fig. 110); this layer very soon divides periclinally into two.

Similar results are naturally to be obtained from transverse sections (fig. 111); in these the trabeculæ are seen radiating, as it were, from the slightly convex, sub-archesporial pad, which intervenes between the sporogenous tissue and the vascular bundle of the sporophyll. This figure is added for purposes of comparison with *Lycopodium*, and, if we refer back to fig. 43 of *L. Selago* or fig. 64 of *L. clavatum*, the closeness of the similarity between them will be sufficiently plain. The potential archesporium of *Isoetes* clearly corresponds to the curved sporogenous mass of *Lyco-podium*, the chief difference lying in the differentiation of the former into sterile trabeculæ and sporogenous masses. In fact, if we imagine a heterosporous Lycopod with its sporangium widened out radially, and its enlarged sporogenous mass partly sterilized so as to form trabeculæ, the result would be practically what is seen in *Isoetes lacustris*.

It is often wrongly assumed that the sporangia of *Isoetes* are actually partitioned, and some of the published drawings, if not corrected by description, support this error. The trabeculæ are not partitions, but, as their name implies, rods of tissue which radiate upwards from the subarchesporial pad; they are frequently very irregular both in number and form; though, in our figs. 110 and 111, more regular examples have been chosen, the specimen drawn as fig. 112 will sufficiently show such irregular branchings as may occur, while the structure of them is displayed more in detail in fig. 113; here the spore-mother-cells have separated from one another and rounded off: the tissues forming the trabeculæ have differentiated into a superficial tapetum, here shaded, and central parts which remain after the tapetum becomes disorganized. As the sporangia approach maturity all that remains of the trabeculæ is the shrivelled central part, from which, as the spores ripen, the cell contents are abstracted, so that even the cell-structure is difficult to make out (fig. 115). In this state a comparison may be made with what has already been seen in *Lepidodendron*; if fig. 115 of *Isoetes* be put side by side with fig. 102 of *Lepidodendron*, the similarity is excessively striking. I think the conclusion may fairly be drawn that the processes observed in the sporangium of *Lepidodendron* are essentially similar to the trabeculæ of *Isoetes*, though we note differences of detail in their distribution in the two cases, and though we are not in a position to state that their development in *Lepidodendron* is like that in *Isoetes*.

When further the form of the sporangium in the two cases is compared, especially the large area and radial extension, it may be concluded that *Lepidodendron* presents characters of the sporangium more closely similar to those of *Isoetes* than does any one

of the living Lycopods; this comparison materially strengthens the affinity of *Isoetes* with the Lycopodinæ.

The description above given relates both in *Lepidodendron* and in *Isoetes* to the microsporangium; as regards the megasporangium of the latter plant, I have nothing material to add to the excellent description of GOEBEL, beyond saying that I am able to confirm his results; the differentiation of the potential archesporium into sterile trabeculæ and fertile spore-mother-cells is clearly similar to that in the microsporangium. In both cases a partial sterilization of the potential archesporium is to be traced.

Summary of Results from Study of Isoetes.

(1.) The sporangium of *Isoetes* corresponds in position on the sporophyll to that of the Lycopods, in form it compares more nearly with *Lepidodendron*.

(2.) It originates from superficial cells of the basal part of the sporophyll, which divide periclinally and anticlinally, forming the superficial wall, and subjacent archesporium.

(3.) Additions appear to be sometimes made to the sporogenous tissue by subsequent periclinal divisions of superficial cells, as in *Equisetum*, and occasionally in *Selaginella* and *Lycopodium*.

(4.) The sporogenous tissue is later differentiated into sterile trabeculæ and spore-producing masses; the former are derived by sterilization of potential archesporial cells.

(5.) The trabeculæ resemble in structure and function those of *Lepidostrobus Brownii*.

THEORETICAL CONSIDERATION OF THE RESULTS ACQUIRED BY STUDY OF THE LIVING LYCOPODS, LEPIDODENDRON AND ISOETES.

We have now examined a considerable number of forms, living and fossil, which are more or less closely allied to one another, and may venture upon some theoretical conclusions which may be drawn from the study of them. It is, meanwhile, to be remembered that such organisms as these which have been studied are generally believed to represent very ancient types; this may be concluded both on comparative and on palæontological grounds.

It was assumed at the outset that, other things being equal, it is a distinct advantage to an organism to increase the number of spores produced; we may specially examine these plants from this point of view, and consider how they severally illustrate the balance of two conflicting factors, viz., (1) the advantage of increased spore-production, (2) the risk of damage, and the difficulty of nutrition of large masses of sporogenous tissue, especially at the period of the tetrad-division, when the cells of the sporogenous mass do not form a coherent and firm tissue.

For reasons above stated (p. 506), *Phylloglossum* is regarded as a primitive, rather than a reduced form ; its small and comparatively simple strobilus is, on our working hypothesis, the counterpart of a sporogonial head, which in this plant is separated sharply by the intercalary growth of the axis, from the protocorm with its proto-phylls. The sporangium of *Phylloglossum* is not in any sense an extreme type, either as regards size, or peculiarity of form ; it seems not improbable that it may represent something like the original Lycopodinous sporangium, though there is no definite proof that it is the original type.

Supposing it to be so, and its whole strobilus to be really a primitive type, let us imagine in what ways the increase in spore-production might be effected, and then inquire whether any of these are exemplified by plants before us. We may imagine that the spore-production might be increased :—

(1.) By lengthening and even branching of the strobilus, and increase of the number of sporophylls and sporangia produced in ordinary sequence.

(2.) By increase in size of the individual sporangium.

(3.) By formation of adventitious sporangia in places where they were not pre-viously produced.

We will consider each of these separately.

(3.) The third head may be at once dismissed ; within the Lycopods and allied forms which we are considering, no adventitious sporangia have been observed.

1. Lengthening and branching of the strobilus has probably been a potent factor in the production of the large Lycopodinous forms from a simpler ancestry ;* whether or not *Phylloglossum* really represents, or is at all like such ancestors, it is not to be doubted that earlier ancestors were simpler than they, and it has been already remarked that the structure and development of its strobilus show facts not incom-patible with the recognition of its strobilus as the counterpart of a sporogonial head. It is not difficult to realize how a strobilus, gifted with continued apical growth, and a power of branching (as is foreshadowed in the sporangia of some Bryophytes as a rare abnormality, and also is seen in fig. 23 in *Phylloglossum*) might form a larger number of sporangia than its predecessors, and the total output of spores be thus increased. But to nourish the increasing number of spores, increased vegetative development will be needed : this need is not met in the Lycopods by increase of the vegetative development of the sexual, but of the non-sexual generation, and on com-parative grounds it appears to me probable that an increased assimilative power was acquired by them in the following most interesting way. It is a familiar fact that certain species of *Lycopodium* have alternating sterile and fertile zones ; examining the limits of the fertile zones, the sporangia, though present, are abortive ; in the presence of these arrested sporangia I believe that we have evidence that the whole sequence of sterile and fertile zones is the result of partial sterilization of a primitive strobilus, all potentially fertile, but of which parts are sterilized, and carry on merely

* See footnote, p. 484.

a vegetative function; the evidence of its potential fertility is to be found in the sporangia present in the axils of many of the vegetative leaves, though arrested in their development at a very early stage (*e.g.*, in *L. Selago, hippuris, dichotomum, carinatum*). In such species there is thus seen a primitive and incomplete differentiation of vegetative from sporogenous parts, and I see no improbability, but rather all evidence in favour of the view that the former are the result of sterilization of the latter. The physiological advantage is too obvious to need lengthy explanation, while, as the apex of the axis in these species retains its power of continued growth, the production of spores may be carried on without definite limit.

In other species the differentiation of the sterile and fertile zones is more complete, the latter appearing as clearly defined strobili (*e.g.*, *L. alpinum, clavatum; Selaginella Martensii, spinosa*). It has been repeatedly noted in the above pages that arrested sporangia are present at the base of the strobilus of such plants, they are also found at the apex. In the latter position they would be generally accepted as potential sporangia, arrested owing to insufficient supply of nourishment. I now suggest that the arrested sporangia at the base of the strobilus are to be explained in the same way, though they bridge over the limit between the sporophyll and the true foliage leaf. The correlation of growth is often very marked, *e.g.*, in *L. phlegmaria*, where the foliage leaves are relatively large, while the sporophylls are small: such forms might be regarded as those most advanced in point of differentiation.

I see no other way of explaining the presence of these abortive sporangia at the base of the strobilus, unless they be accepted as "prophetic germs," a suggestion which will not readily commend itself. And thus the general conclusion may be approached, which would well explain the facts, though it must only be held as an hypothesis, viz., *that, exclusive of the protocorm and protophylls, the plant of Lycopodium or of Selaginella may be looked upon as the result of elaboration of a strobilus by continued apical growth and branching;* that parts of the strobilus (usually the basal part, but sometimes alternating zones) became sterilized, the sporangia arrested, or entirely aborted, and these parts carry on the assimilating function, and supply nourishment to the residuary, fertile portions; these, in modern Lycopodia, appearing as the recurring fertile zones or as distinct strobili. Briefly put, *we see in Lycopodium evidence that the ordinary foliage leaf is a sterilized sporophyll.*

There is, I think, no inherent improbability in this theory, while it explains the presence of the arrested sporangia, which would otherwise be unintelligible. We may now picture to ourselves how, from a simple form, such as *Phylloglossum*, the larger Lycopods may have arisen by elongation and branching of the strobilus, and increase in number of sporophylls and sporangia, and, further, by partial sterilization of the strobilus.

As an objection to this theory it may be urged that it implies an antithesis between the protophylls and the ordinary vegetative leaves; that antithesis is, however, sufficiently clear in *Phylloglossum*, which, on other grounds, is marked out as

being probably a primitive type of Lycopod. I do not wish, however, to press this antithesis too far; if we keep in view, for purposes of comparison, the case of the Bryophyta, it will be remembered that the distinction between the seta and the capsule is not always a very distinct one, and this may also have been the case in the progenitors of the Lycopodinous series.

2. Having now considered the first means by which greater spore-production might be promoted, viz., by increase in number of sporangia produced in ordinary sequence, and having seen that it has probably been exemplified in *Lycopodium* and *Selaginella*, we may consider the second, viz., *increase in size of the individual sporangium*. This may involve any one or all of the dimensions* of the sporangium; *e.g.*, the sporogenous tissue might be extended (*a*) in a radial direction, as regards the whole strobilus, or (*b*) in a tangential, or (*c*) it might be deepened, while retaining the same area on the sporophyll; or there may be various combinations of (*a*), (*b*), and (*c*).

Within the genera, *Lycopodium* and *Selaginella*, the *depth* (*c*) of the sporogenous mass remains more constant than the other dimensions of the sporangium, and the archesporium is, with the exception of a few abnormal cases, defined by the first periclinal wall. There is apparently a physiological explanation of the fact that the depth of the sporogenous mass is almost uniform; the limit is probably imposed by the difficulty of transmission of nutritive materials upwards from the sub-archesporial pad throughout the developing sporogenous mass: this point will not therefore be considered further at present.

The radial (*a*) and tangential (*b*) dimensions are, however, less constant, and the fluctuations in the genera in question are of importance as leading to a comparison with other forms.

Taking first (*a*), the radial dimension, we see in *Phylloglossum*, and in *L. Selago*, and others, that the sporogenous tissue is referable in the radial section to a single cell, and these species have a comparatively narrow, radially compressed sporangium; in other species, the base of the sporangium is broader, and the sporogenous tissue is referable to *two* (*L. inundatum* and *Selaginella*) or even to three cells (*L. clavatum* and *alpinum*) in each radial section. The fluctuations, which are thus comparatively trifling in *Lycopodium*, acquire a new interest when the sporangia of *Isoetes* and of *Lepidodendron* are compared. In the former, the mature sporangium is rather like an oval cake, with its major axis in the radial direction, the sporangium not being radially compressed as in *Lycopodium*. Obviously here is an increased accommodation for spore-production as compared with *Lycopodium*, brought about by increase of the radial dimension. The development bears this out, for the archesporium is

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* It is to be noted that the comparison is not based upon absolute measurement, which might be misleading, but rather upon tissue-complexity; one spore-mother-cell, though a small one, will give rise to four spores, which, though small, may yet serve to produce four new individuals, just as well as four large ones.

referable to a considerable number of cells in each radial section. Finally, in *Lepido-dendron*, this radial extension attains its maximum, the mature sporangia being more than three times as long (radially) as they are broad (tangentially), and the possibility of production of large numbers of spores is thus greatly increased.

Turning now to (*b*), the tangential dimensions, considerable fluctuations are to be found in our series. Perhaps the simplest case is that of *L. phlegmaria*, where the sporogenous tissue appears to be referable in tangential section to *two* cells. Passing to *Selaginella spinosa*, it is referable to *three* or *four*; in *L. Selago*, it is referable to about *six*; in *L. clavatum* and *alpinum*, about twelve. These figures, relating as they do to early stages of development, give, nevertheless, a clue to the final form and dimensions of the sporangia : while the sporangium of *Selaginella* is very little extended tangentially, and hardly shows any trace of the kidney-like form, that of *L. clavatum* assumes the form of an inverted U (fig. 92, *c*, *c*). The behaviour of the sub-archesporial pad is also worthy of note, since, in such forms as the last, it attains a very considerable degree of development, and projects far upwards into the cavity of the sporangium.

It is thus seen that the various dimensions of the sporangium are susceptible of fluctuations, in different genera and species, and, as one or more of the dimensions is increased, the individual sporangium may attain, when mature, a very considerably increased size, *e.g.*, those of *Isoetes* and *Lepidodendron*. But when this is the case, the enlarged sac is exposed, both to risks of damage from without and to difficulties from within in the supply of nourishment to the large mass of developing spores. It is, doubtless, these two factors which have led to those peculiar developments known as the trabeculæ of *Isoetes*, and the somewhat similar growths now described for *Lepido-dendron*. In neither of these plants do the sporangia appear to be completely partitioned ; but bands, or plates of sterile tissue (trabeculæ) spring from the sub-archesporial pad, and pass upwards through the sporogenous tissue, and, at least in *Isoetes*, are continuous to the upper wall of the sporangium, but it has not been possible to prove this continuity in the case of *Lepidodendron*. Small upgrowths of a somewhat similar nature have been found at times in *L. clavatum* and *alpinum*. The physiological importance of these is plain ; they probably serve as channels for conveyance of nutritive materials into the very mass of developing spores, and they increase the available surface of such transmission. It is specially interesting to note their presence in those Lycopod-sporangia which are the largest, and most extended in a radial direction.

In *L. clavatum* and *alpinum* (and, perhaps, also in *Lepidodendron*) the trabeculæ appear to be mere upgrowths from the sub-archesporial pad. In *Isoetes*, however, it was shown by GOEBEL, and now amply verified, that they are formed by sterilization of part of a potential archesporium ; here they are continuous to the upper wall, and probably serve also a mechanical purpose. It has been suggested that they serve as props of the wall at the time when the sporogenous mass is semi-fluid (FARMER,

'Annals of Botany,' vol. 5, p. 49); they may so act, but the peculiar dimpled appearance, often seen in the upper surface of a microsporangium, would rather suggest that they act as stays, in fact similarly to the trabeculæ of *Caulerpa*, as suggested by JANSE ('Pringsh. Jahrb.,' vol. 21, p. 272), and are, at the critical period, in a state of tension.

In *Isoetes* the trabeculæ are not uncommonly connected either by their branchlets (figs. 112, 113), or laterally along a considerable distance so as to form plates, which may also be connected with the walls. Such appearances suggest an approach to a partitioned state of the sporangium, which, however, has not been found in any of the plants hitherto considered. The advantages of a partitioned condition, where the sporangium is large, are plain enough; not only would the nutritive surface adjoining the developing spore be enlarged, but mechanical strength would be afforded to the large developing sac, and, finally, while a single puncture of the large non-septate sporangium by animal, or other agency, would probably destroy the whole mass of spores, if the sporangium were septate only one compartment would suffer. It is clear then that where the size of sporangia is larger, the advantage of a septate condition will become greater. These considerations, together with the facts drawn from *Isoetes*, suggest the question whether such partitioned sporangia of the Lycopodinous type occur, and, if so, in what plants?

A striking answer to this question is, I believe, to be found in a genus of Lycopodineous affinity, in which the sporangium is extended in the radial direction. We have already noted fluctuations in this dimension within the genus *Lycopodium* (compare figs. 36, 58, 73), while *Isoetes* in which the potential archesporium is partially sterilized to form the trabeculæ, is a still more pronounced example among living plants. As will now be explained at length, it is in *Tmesipteris* that we appear to find a comparatively simple septate sporangium, or synangium—which, on the ground of the above considerations, appears to be a more efficient type of construction than such trabecular sporangia as are seen in *Isoetes* or *Lepidodendron*.

PSILOTACEÆ.

The Psilotaceæ are a family which occupies a place somewhat apart from other Lycopodineæ, though they are usually classed with them; their conformation is in many ways distinct from that of other forms, while the two genera of the family, viz., *Psilotum* and *Tmesipteris*, show fundamental similarity to one another, though sufficiently distinct in details.

It is probably owing to their somewhat separate position, and their marked peculiarities, that they have been the subject of frequent discussion, both from the purely morphological, and also from the systematic point of view. The morphological discussions have chiefly centred round the spore-bearing members (synangia or sporangiophores); it is unnecessary to criticise in detail the views which are, or

have been held, for this has already been done most admirably by Solms-Laubach ('Ann. d. Jard. Bot. d. Buitenzorg,' 4, p. 139, &c.), while a very complete list of literature up to the date of his writing is given, together with brief critical remarks, as an appendix to his memoir (*loc cit.*, p. 187). Since then further observations have been made by M. Dangeard ('Le Botaniste,' série 2, May, 1891), and by Mr. Vaughan Jennings ('Proc. Roy. Irish Acad.,' series III., vol. 2, 1891), but their statements do not materially alter the position, or determine the points at issue. It is to be remarked that, notwithstanding all that has been written, the knowledge of the facts of development of the peculiar spore-bearing members is still incomplete; their mature structure has been described and figured with all necessary detail by M. Bertrand ('Arch. Bot. du Nord.,' vol. 1, p. 457, for *Psilotum*, and p. 528 for *Tmesipteris*), and others; the external form of the developing organs has been beautifully illustrated for *Psilotum* by Solms-Laubach (*loc. cit.*, Plate 23), and by Bertrand (*loc. cit.*, p. 462, fig. 199); drawings have also been made of the young sporangiophores of *Tmesipteris* by Goebel ('Bot. Zeit.,' 1881, Plate 6, fig. 12), and by Jennings: but the internal details are very incompletely known, the only accounts hitherto published being those of Juraniyi ('Bot. Zeit.,' 1871), which is not illustrated, and of Goebel ('Bot. Zeit.,' 1881, p. 688, Plate 6, figs. 9–12). Thus there is need for the subject to be taken up afresh, and the internal details of development to be described, before the morphological question can be finally decided.

The chief views as to the nature of the sporangiophores are these : (1) that the whole sporangiophore is a single appendicular (foliar) member, (2) that it is a structure of reduced type, consisting of an axis bearing a terminal synangium, and two leaves.

The former view (1) was held by all the older morphologists; it was accepted by Mettenius ('Bot. Zeit.,' 1867, p. 98), and by Luerssen, and has been most ably defended, on the basis of observations of external form during development, by Graf Solms (*loc. cit.*). The second view was propounded by Juraniyi ('Bot. Zeit.,' 1871, p. 177), and has been adopted by Goebel on the basis of his own observations ('Bot. Zeit.,' 1881, p. 689). The arguments have turned upon the position of the organic apex of the whole lateral structure, and the relations of the other parts to it in point of time and place of their first appearance. Obviously, this may be determined either by external observation or by histological analysis of the developing parts, but best by a combination of both methods.

Juraniyi and Goebel state that the synangium is actually terminal on the sporangiophore, and this view is very distinctly stated also by M. Bertrand ('Arch. Bot. du Nord,' vol. 1, p. 468). Graf Solms, however (*loc. cit.*, p. 180, and Plate 23), has also described and illustrated the external characters for *Psilotum*, while, at the same time, he suitably criticises the results of other observers which differ from his own. He finds that the sporangiophore first makes its appearance as a lateral flat extension of the growing point of the shoot, which is soon separated

from the latter by a very shallow groove : at this time the young sporangiophore has a tongue-like form, being slightly channelled on the upper surface. In the middle of this slight median channel a flat upgrowth appears, and this is the young synangium, which thus arises, according to SOLMS, from the upper surface of the sporangiophore. On the basis of these observations he maintains the older foliar view of the sporangiophore.

Our purpose will now be to see how far the study of radial sections, traversing the apex of the axis and the very young sporangiophores, will bear out this account of the development. Of the two genera the parts in *Psilotum* are more shortly stalked in the mature state than those of *Tmesipteris :* the leaf-lobes of the latter are larger, while the loculi of the synangium, being only two, and both in the median plane, this plant is clearly the one which will more readily yield results from longitudinal sections. *Psilotum*, on the other hand, having a trilocular synangium, will be difficult to work in longitudinal section, and only one of the three loculi can possibly be cut fairly in its median plane in any one section. Accordingly, as both genera have been studied, the description of the results for *Tmesipteris* will be given first. The observations were chiefly made on material supplied by Mr. VAUGHAN JENNINGS ; I have also to acknowledge specimens from Mr. G. M. THOMSON, sent direct from New Zealand, while one fine apical bud was cut by Professor I. BAYLEY BALFOUR from the living plant in the Edinburgh Botanic Garden.

TMESIPTERIS.

The apical cone of the plant is very variable in bulk: in strong young shoots it may be a broad dome (fig. 118), while in weaker specimens, or those in which apical growth is beginning to fail, it may be comparatively narrow. In the large as well as the small specimens a single initial is usually present (*x*, fig. 118), but its segmentation does not appear to be strictly regular, and it is difficult to refer the whole meristem to the activity of one parent cell. This conclusion is borne out by the appearance of the apex as seen from above (fig. 119), when the initial is seen to have the form of a three-sided pyramid, but the tissue around it is not readily to be parcelled out into groups derived from regular segments : secondary initials (cells marked *o*) also occur frequently. It will be noted that these results materially coincide with those obtained by SOLMS for *Psilotum* (*loc. cit.*).

Passing from the actual apex the sides of the cone are covered externally by deep prismatic cells, which are of somewhat irregular origin, depth, and arrangement : when a leaf or sporangiophore is about to be formed certain of these increase in size, and undergo both periclinal and anticlinal divisions so as to form a massive outgrowth (figs. 120–121), the summit of which is occupied, as seen in radial section, by a single larger cell of a wedge-like (fig. 120, 123) or prismatic (fig. 121) form : it is not improbable that the latter passes over to the wedge-like form as the part develops.

A transverse section of the axis passing through such a young leaf does not disclose any marked feature (fig. 122). In these early states I find it impossible to say whether the part in question will be a vegetative leaf or a sporangiophore, and even when older it is still a matter of uncertainty; it is, however, believed that fig. 123 represents a foliage leaf, and is to be so recognized by the narrow form which becomes more pronounced in the older vegetative leaves: those, however, which are to develop as sporangiophores, soon show an increase in thickness, while they grow less in length: an excrescence of the adaxial surface soon becomes apparent (fig. 124), in which the superficial cells are chiefly involved: the lower limit of the tissues resulting from their divisions is shown by a heavy line in figs. 120, 121, 124, and 125, and from a comparison of these it will be plain that, while the essential parts of the synangium are derived from the superficial cells of the young leaf, the subjacent cells also bear a part, forming a sub-archesporial pad (*p.*, fig. 125). The superficial cells at first form a rather regular series (fig. 120), which may be compared with the cells which give rise to the sporangia in *Lycopodium clavatum*, or in *Isoetes*: they undergo more or less regular divisions (fig. 124), which, however, I have been unable to follow in detail: a band of tissue some four or more layers in depth is thus produced. At about this period certain masses of cells assume the characters of a sporogenous tissue (figs. 125, 125 *bis*, shaded cells); but though they can be recognized as such by the character of the cells, it is exceedingly difficult to define the actual limits of these sporogenous masses. The more superficial tissues, as well as the band intervening between the two sporogenous masses remain sterile, the latter developing into the septum, while the former develop into the walls of the synangium: it is specially to be noted that the origin of the tissue of the sterile septum, which separates the sporangia, seems to be similar to that of the sporogenous masses themselves.

I have not been able to decide whether the archesporium is here defined at once by the first periclinal division of the superficial cells (fig. 120), or whether successive additions are made to the sporogenous tissue by subsequent periclinal divisions of superficial cells, as in *Equisetum*, and in a less degree in *Isoetes* and *Selaginella*. I am, however, inclined to think the latter to be the case, since in such examples as that shown in fig. 124, the superficial cells are very deep, while the lower cells are not so.

As the development proceeds, the original arrangement of the cells becomes disturbed by unequal growth (fig. 126); the more superficial layers develop into the rather massive wall, and the cells immediately surrounding the sporogenous masses become compressed, and ultimately disorganized. It has been above noted that it is difficult to recognize with certainty the exact limits of the sporogenous masses in the synangia (compare fig. 126): this is probably due to the fact that there is no very clearly defined tapetum, nor is the whole of the sporogenous mass used up in the actual formation of spores, but a considerable proportion of the cells composing it, acting as a diffused tapetum, become broken down, and disappear in a manner similar to that to be described more in detail in *Psilotum* (p. 549).

Finally, a strand of vascular tissue, of which the origin may be traced in figs. 124, 125, 126, is formed, extending up the sporangiophore ; on entering the synangium, it passes up to the base of the septum, and there branches right and left, the two branch-bundles traversing the margins of the septum (compare figs. 146–148).

When mature, the wall of the synangium consists of a superficial layer of deep cells, with thick cell-walls (figs. 146–148), which are similar to those of the wall of the sporangium of *Lepidostrobus Brownii* (fig. 93), and as in that fossil, so in *Tmesipteris*, a band of thinner-walled compressed cells, three to four layers thick, supports the superficial layer internally (fig. 146). These cells have pitted walls, and are not definitely limited internally, but irregular tatters of cell-wall project into the cavity of the synangium, showing thus that there is no clear limit between the wall of the synangium and the tapetum.

The septum shows in the main a structure similar to this inner band of the wall, with which it is continuous ; it consists of a firm plate of narrow tabular cells, four to six layers in thickness, with profusely pitted, woody walls. The septum is also coated by the remains of thinner-walled disorganized cells. As already noted, the branches of the vascular bundle which enters the synangium pass right and left up the margin of the septum (fig. 148) ; these bundles are seen as bands of tracheides (fig. 146) in transverse sections through the lower part of the septum ; the bundles are not sharply differentiated from the surrounding tissues, and it appears to consist only of xylem. A number of tracheides, continuous with the bundle, extend along the central part of the septum ; and from the position of the bundle, it appears to belong to the septum, rather than the external wall of the synangium. Moreover, it will subsequently be seen that the branch vascular bundles are absent in those abnormal synangia in which the septum is wanting or incomplete.

Turning now to sections in other directions, if a synangium be cut vertically (along a line x, x, fig. 125), the appearance presented is as in fig. 127 ; l, l are the lateral lobes (leaves of some writers), which grow out right and left from the summit of the sporangiophore. The shaded cells are the sporogenous mass, and the arrangement of the walls supports rather than discountenances the view that the archesporium is not defined by the first periclinal division of superficial cells ; it is easy to see the correspondence between figs. 127 and 125.

If the sporangiophore be cut through transversely, the appearance at successive ages would be such as is shown in figs. 128–131. When very young the outline of the section will be oval (fig 128), the lateral lobes not having as yet appeared : the cells adjoining the axis may be recognized as those which will form the sporangium. It must be noted that they are necessarily cut obliquely, as reference to fig. 124 will show ; hence the superficial cells appear shallower than they really are. When rather older (fig. 129) the formation of the leaf-lobes will have begun (l, l), which then proceeds rapidly (fig. 130). Meanwhile the sporangium (in these transverse sections it is the lower of the two loculi of the sporangium which is cut through)

becomes broadly convex, and the steps of the development, as shown in figs. 130, 131, will be seen to coincide with what has been already stated for the radial section.

The above descriptions are based entirely upon the developmental study of normal specimens of *Tmesipteris*; whatever value readers may be disposed to accord to evidence from examination of abnormalities, it is of importance to see what it amounts to, since such evidence has been repeatedly used by previous writers on the Psilotaceæ, though chiefly with reference to *Psilotum*. The importance of such evidence will vary in different cases, according to the frequency of occurrence of any given abnormality: other things being equal, it appears to me that those abnormalities which recur most frequently in a given species will be those which are most worthy of consideration for morphological argument.

As far as I am able to judge from the specimens in my possession, *Tmesipteris* is an unstable plant as regards form. Twenty-four plants have been examined, and upon these were found twenty-six synangia which showed abnormal development—an average of more than one on each plant: they were, however, unequally distributed, some plants bearing no abnormal synangia, others bearing several. It would thus appear that *Tmesipteris* is unusually prone to variation in details of its synangia.

The abnormalities may be considered first from the point of view of external form: it has been a matter of frequent observation that "double leaves" occur in *Psilotum* without a synangium: SOLMS remarks, however (*loc. cit.*, p. 175), that the rudiment of a synangium may almost always be detected in such cases in the usual position. An example of this is shown in fig. 149, for *Tmesipteris*, where the abortive synangium (*sy.*) is seen in the normal position: it is to be noted that it is placed on the adaxial face, and below the indentation between the leaf-lobes. This is then a simple case of arrest of the whole synangium. Similar specimens of *Tmesipteris* have been figured and described by M. BERTRAND ('Arch. Bot. d. Nord,' vol. 1, p. 475). Either the upper or the lower lobe of the synangium may be arrested, while the other lobe develops in the usual way; these two cases are shown in figs. 150, 151; such examples of arrest of development of one loculus are to be carefully distinguished from cases to be described below, showing, in various degrees, the disappearance of the septum between the loculi. A correlative vegetative growth, following arrest of the synangium, was rarely found: in fig. 152, however, a long process is seen bearing two small lobes; this arises in place of the synangium, and is clearly seated, as before, on the adaxial face of the sporangiophore. A different form of correlative growth is seen in fig. 153: a "double-leaf" of abnormal form is here shown, in which the lower part has virtually the form of the single foliage leaf: seated on the adaxial face, and near its base, is a small brown process, which resembles an abortive synangium (*sy.*), while the upper part assumes the character of the "double-leaf," but with the lobes partially coherent. I have not seen in *Tmesipteris* any case of appearance of a third lobe, as described by Solms for *Psilotum* (*loc. cit.*, p. 175, Plate 23, fig. 8). In the abnormalities thus described, I see nothing inconsistent with the hypothesis above put forward: that

described last (fig. 153) appears decidedly to support the suggestion, based on study of the normal development, that the sporangiophore is a single leaf with two lobes, bearing the synangium on its adaxial face.

It is well known that both *Psilotum* and *Tmesipteris* show alternating sterile and fertile portions of the same shoot, similar to those seen in *Lycopodium Selago*, and other species. It is at the upper or lower limits of these fertile zones that abnormalities are most frequent, and even sporangiophores, which are otherwise normal, show there great variations in vegetative development; thus, fig. 154 represents a sporangiophore from the middle of a fertile zone, with fully developed leaf-lobes, while fig. 155 represents one of similar age from the upper limit of the fertile zone; here the leaf-lobes are small and stunted, though the synangium shows its normal characters. The synangium itself is, however, liable to variations in form of a somewhat parallel nature, involving (i.) greater complexity, or (ii.) simplification as compared with the normal. In two specimens from the middle of the fertile zone, a trilocular synangium was seen (fig. 156), the position of the three loculi being similar to those in *Psilotum*, with which the correspondence of the whole sporangiophore is in these exceptional cases very close. Fig. 157 represents a less regular case where three loculi are seated on one sporangiophore.

But the greatest interest in connection with the hypothesis above put forward is centred in those abnormal synangia which show *simplification* of external form, and it will presently be shown that simplification of internal structure follows that of form : such simpler synangia are most frequently, though not exclusively, found at the limits of the fertile zone, or on specimens which have developed weakly. The most frequent simplification is found in the absence of the groove separating the two lobes of the synangium, so that externally the synangium appears as a single boat-shaped body (fig. 158). In others the form may be shorter (fig. 159), but in such specimens, of which the internal structure will be seen to present facts of the greatest importance for our theory, it is to be noted that the two projecting points, as well as the slight median groove, show that the whole body represents a complete synangium of a reduced type ; it is not to be ascribed to the arrest of one or other of the loculi, as in figs. 150 or 151. Finally, in fig. 160, we see an extreme case of reduction, the synangium being here represented by a small spherical body, borne in the usual position on a sporangiophore, of which the leaf-lobes are of a very small size. The above examples will show the chief lines of modification of form to which the synangia are liable ; but the more direct interest in connection with our hypothesis is to be found in the modifications of internal structure and development which accompany them.

The normal structure of the synangium and its development have been described in detail above (p. 541–543) ; examination of sections of such synangia as those we have just been discussing shows considerable deviations from the normal structure, especially as regards the partial or even complete abortion of the septum ; these deviations have a certain relation to the external form. The structural details to be now described

have been obtained by the comparison of sections of fourteen specimens of different ages. Those of the type shown in fig. 158 deviate the least from the normal in form, and in section these sometimes show a complete septum, of apparently normal structure; of the others, however, which were approaching maturity, one showed only a slight flange, projecting inwards into the single large cavity; this doubtless represents the margin of the septum, of which the central part has disappeared, *in other specimens there was no representative of the septum at all.* From the form of these synangia, as well as from the occasional presence of vestiges of the septum, we learn that they are not due to abortion of one-half of the synangium, but that they represent the whole synangium, and they thus demonstrate *that the septum may be partially or completely abortive.* The same was found to be the case in the specimen shown in fig. 160, but the best results have been obtained from those of the type shown in fig. 159, for several of these of different ages were obtained, sufficient indeed to supply the most essential features in the development of these non-septate synangia. It has already been shown in the development of normal synangia, that it is impossible in early stages to differentiate the sporogenous tissue from the septum, and that in later phases the limit was not clearly marked (see p. 542); in the non-septate synangia the distinction never appears, or, at most, it is only slightly suggested. Such a synangium in the young state is shown in fig. 161, from which it will be seen that the form of the synangium is of the type of fig. 150, the groove between the lobes being almost obsolete; the vascular bundle stops short at the base of the synangium, instead of passing far into it, as described for the normal type; the place where the septum should be is indicated by a slight flange of firmer tissue projecting downwards from the upper wall (figs. 161, 162), but instead of continuing downwards as firm tissue, as in the normal synangia, it merges into a mass of cells which fill the synangium, and consists of (i.) cells with less dense contents (the tapetum, *t*, fig. 162), and (ii.) more densely protoplasmic cells (the sporogenous cells, *s*, fig. 162); the former appear as a peripheral band (*t, t*), while the latter (*s, s*) occupy the centre. The place where the septum should be demands special notice: there the tissue is disposed, roughly speaking, in rows, as in the normal synangium; the tissue opposite the projecting flange of the incomplete septum appears to be sometimes of the tapetal character, and disappears as the synangium develops (fig. 163). *But its cells may also be sporogenous* (fig. 162); in the younger state they may be so recognized by their denser protoplasmic contents, as in the figure above quoted, but the best demonstration of this fact is afforded by later stages, where the sporogenous cells are more clearly distinguished, by their definite cell-walls and nuclei, from the tapetal cells which are undergoing disorganization. Fig. 164 shows a whole synangium of the same type as fig. 159, cut in median section; there is hardly any groove defining the two halves of the synangium, and no clear indication of the septum internally, while the cavity is occupied partly by disorganized cells of the peripheral and diffused tapetum, partly by the sporogenous

cells, which hang together in connected masses. In fig. 165 part of the contents of this synangium, lying about the centre of it, are represented under a high power, while the line (x, x) shows the position which the septum should normally occupy at present. Now it is plain that a connected thread of sporogenous cells, with definite cell-walls and nuclei, which make them readily distinguishable from the tapetal cells, extends quite across that line; thus it is demonstrated that *the tissue which would normally develop as the septum, may, on occasions, develop as tapetum, or even as sporogenous tissue.*

Figs. 166–168 illustrate other examples of a similar nature, which were also of size far below the normal. Figs. 166, 167 show two transverse sections of a small synangium, the first near its base, the second nearer to its upper surface. From these it will be seen that a *partial septum* was present (*s.*), which projects from the upper wall of the synangium for a certain distance downwards into the cavity, but there stops short. Finally, fig. 168 represents a transverse section through the small spherical synangium shown in fig. 160, and in it no trace of a septum was found, the single cavity being occupied by immature spores (*sp.*).

The modification of the vascular system of the synangium where the septum is absent is worthy of mention. It has already been stated that in normal synangia a vascular bundle enters the stalk, and that strands, consisting chiefly of tracheides, branch right and left, entering the septum, and running along its margin about four layers of cells from the outer surface. In synangia, where the septum is incomplete or absent, I find no such septal bundles; in these cases the vascular supply seems to stop short at the base. This behaviour of the septal bundles shows an obvious correlation with the complexity of structure of the whole synangium.

The details described in the above paragraphs occur in synangia, which will commonly be designated "abnormal." In so far as they differ from the common type, they are rightly so called; but it is to be remarked that there is some method in their abnormality; their occurrence, especially at the limits of the fertile zones, the frequency of their appearance, and the correlation of smaller size and greater simplicity of external form of the synangium with the imperfect development, or even entire absence of the septum, all point to the conclusion that this is not a case of haphazard monstrosity. The whole series appears to me to illustrate within the one species what has been recognized elsewhere for distinct genera, such as *Lycopodium, Isoetes,* and *Lepidodendron.* *Where the sporangium is large, sterile bands of tissue are present, while in smaller sporangia homologous with these, sterile bands of tissue may be entirely absent. But whereas in the other cases quoted the sterile tissue was represented only by incomplete trabeculœ, in* Tmesipteris *the same rule is found to apply to the complete septum.*

It may doubtless be objected that these smaller synangia with simpler structure illustrate the possibility of *fusion* of sporangia normally distinct, rather than that they have any bearing on the question of formation of septa; in fact, that they are extreme cases of a *progressive reduction* in a plant which is on the down grade of

morphological change, such as has been suggested by STRASBURGER ('Bot. Zeit.,' 1873), and not cases of individual retrogression in a plant which is, as I should suppose, on the up-grade. But when we look at the whole question from the point of view of increase of number of spores, and compare *Tmesipteris*, *Lepidodendron*, and *Lycopodium*, if the line of advance were such as I suggest, it is just at the limits of the fertile zone that individual retrogression to simpler types would be expected, and the physiological explanation of their occurrence would be the running short of nutritive supply, from which would follow the development of synangia of smaller size and less complex structure.

The results from the study of the development in *Tmesipteris* may be summarized as follows :—

1. In their earliest stages the foliage leaves are not readily distinguishable from the sporangiophores either in form or in internal structure, and they occupy a similar position to them upon the axis.

2. In either case a prismatic or wedge-shaped cell occupies the apex, as seen in radial section, but all the tissues are not readily referable to the segmentation of a single cell.

3. The first appearance of the synangium is as an upgrowth of superficial cells of the adaxial face, immediately below the apex of the sporangiophore; cells of the abaxial side also grow strongly, while the apex itself does not grow ; so that the organic apex is soon sunk in the groove between these stronger growths.

4. The superficial cells of the adaxial surface, which are to form the synangium, undergo periclinal and anticlinal divisions, so as to form about four layers; from these are differentiated : (*a*) two sporogenous masses, (*b*) a septum between them, (*c*) the superficial wall.

5. The limits of the sporogenous masses are difficult to define ; this is owing to the fact that there is no definite tapetum, while many of the cells of the sporogenous tissue also become disorganized without undergoing the tetrad division.

6. The tissue of the septum is similar, as regards origin, to the sporogenous masses ; it is therefore possible to regard it as a sterilized portion of a potential archesporium.

7. The lateral leaf-lobes begin to be formed almost simultaneously with the synangium.

8. In synangia of abnormally simple form the septum may be partially or completely abortive.

9. The tissue which normally develops as the septum, may on occasions develop as tapetum, or even as sporogenous tissue.

PSILOTUM.

JURANYI ('Bot. Zeit.,' 1871, p. 177) appears to have been the first to investigate the internal details of development of the sporangiophore in *Psilotum*; he describes

it, when young, as having all the characters of an axial papilla, noting the presence of a cambial strand, which he stated to be absent from the foliage leaf. In this, however, he was mistaken according to Solms (*loc. cit.*, p. 184). He admits the close similarity of the sterile and fertile parts while young, as regards their external form, a point which has above been specially noted for *Tmesipteris*. He describes the synangium as occupying the apex of the lateral appendage or sporangiophore, while a three-sided pyramidal initial cell is present with definite segmentation. Strasburger denies the last facts ('Bot. Zeit.,' 1873, p. 92), and Goebel agrees that an initial cell is not present. He maintains, however, that the synangium is of terminal origin on the sporangiophore, and describes the sporogenous tissue of each loculus as referable to a single archesporial cell, but this single cell he has not observed ('Bot. Zeit.,' 1881, p. 692). The tapetum appears to originate from the sporogenous tissue. The above are the chief results obtained by previous investigators on the internal details of early development in *Psilotum*.

The detailed study of the synangium of *Psilotum* by means of sections is more difficult than that of *Tmesipteris* on account of its tri-locular character. In radial sections through the terminal bud, the young sporangiophores are found to present a general outline and structure similar to those of *Tmesipteris* (compare figs. 134, 136). Fig. 134 shows one such : the cell (×) is believed to represent the organic apex of the sporangiophore, though it is doubtful whether it be this initial which gives rise to the whole mass of the tissue. The synangium thus appears as an outgrowth of the upper surface of the sporangiophore, while the tissue on the abaxial side of it is already growing out into a bulky projection, as has been noted by Goebel (*loc. cit.*, p. 693), and as already seen in *Tmesipteris*. But I have not been able to trace the development of the essential parts of the loculi of the synangium from the superficial cells of the adaxial side of the sporangiophore in this case with the same certainty as in *Tmesipteris*: I think this is chiefly owing to the stalk being here narrower, and to the fact that only one loculus of the synangium can be cut in a median direction in any one section ; supposing this to be the median plane of the whole sporangiophore, then it will be the abaxial loculus which will be thus traversed. And here it may be noted that Goebel's fig. 11 (*loc. cit.*) does not appear to traverse either of the two loculi exactly in a radial plane ; if this were so, the two loculi could not appear so nearly equal in size, and accordingly as both are traversed more or less obliquely the results from the section must be accepted with reservation. A truly radial section of a young synangium is shown in fig. 135, the arrow indicating the direction of the main axis ; the cell (×) in figs. 135, 137, is a conical cell, which is commonly, though perhaps not constantly found occupying the centre of the apical surface of the synangium (compare figs. 136, 139, 141). Divergent statements of other writers have been above noted as relating to the presence or absence of an initial cell in the synangium of *Psilotum*. Juranyi ('Bot. Zeit.,' 1871, p. 179), describes the synangium as growing with an initial cell of the form of a three-sided

pyramid. STRASBURGER ('Bot. Zeit.,' 1873, No. 6), and GOEBEL ('Bot. Zeit.,' 1881, p. 692, and fig. 9, *sp*.) deny that there is one. I can confidently state that a small three-sided pyramidal cell is commonly present in my preparations (× figs. 135, 137), though I am not disposed to assert that this is the primary parent cell of the whole synangium. The cell shaded in fig. 135 is believed to be the archesporial cell for one of the loculi, but after comparison of a large number of sections I am still uncertain whether the whole of the sporogenous tissue in each loculus is really referable to a single parent cell, for just the same difficulty arises here as in *Tmesipteris*, in recognizing the exact limits of the sporogenous masses; here again, this is probably due to the facts (1) that there is no clearly defined tapetum, and (2) that only a part of the sporogenous tissue actually forms spores. The Psilotaceæ are in this respect the most difficult family of the Vascular Cryptogams.

The subsequent stages of development are illustrated by figs. 136–138, and it will be seen from these how the sporogenous masses assume large dimensions, and are at first composed of uniform cells. The wall of the synangium meanwhile becomes multiseriate, and the cells of the outermost layer assume a deep and prismatic form, while the inner layers are narrow. The same is the character of the more superficial cells of the sporogenous mass (fig. 138), so that it is almost impossible to recognize the limit between the tissue of the wall and of the sporogenous mass; the superficial portions of the latter become disorganized without the formation of spores, but there is no clearly defined tapetum. Such is also the fate of a considerable proportion of the more central cells. As the synangium develops, irregular groups of cells of the sporogenous masses assume dense granular contents, and subdivide, while the others remain paler, with more watery contents, and do not divide; the former undergo the final tetrad division, and form spores, while the latter become disorganized. The actual state of partial disorganization is shown in figs. 143, 144; thus, a partial sterilization of cells of a sporogenous tissue, essentially similar to that demonstrated in *Equisetum*, is seen also in *Psilotum*, and, as above stated, it exists also in *Tmesipteris*. This has already been noted by STRASBURGER.

The fan-like tracery of the cells, as seen in radial section of the synangium, shows that the study of transverse sections will present difficulties; these are least in the youngest stages, such as that shown in fig. 139, which corresponds in age nearly to that of fig. 135. The cells shaded are believed to be the archesporia, but I should be slow to make precise statements on this point, and especially so in face of the difficulty, above noted, of assigning definite limits to the sporogenous tissue in older synangia. Further stages of development are shown in figs. 140–142; and when allowance is made for the transverse sections cutting the cell-rows rather obliquely (as must be the case in parts with a fan-like tracery), it will be seen that the transverse sections fit with all reasonable closeness of comparison with the results from the longitudinal.

The main facts derived from the study of *Psilotum* thus coincide with those arrived

at in *Tmesipteris,* as regards the origin of the sporangiophore, and the appearance of the synangium on its upper surface, below the extreme apex. The following points may be noted as more specially applicable to *Psilotum,* in addition to those already summarized for *Tmesipteris :—*

1. The organic apex of the sporangiophore coincides with the groove between the synangium and the pair of abaxial growths (leaf-lobes), as seen in the mature state.

2. A three-sided pyramidal cell is usually present at the apex of the synangium.

3. Each sporogenous mass appears to be referable in origin to a single parent cell, but this has not been actually proved, it being difficult to assign sharp limits to the sporogenous masses.

4. The inner cells of the wall, as well as the superficial cells of the sporogenous masses, become disorganized, but there is no definitely distinguished tapetum.

5. Certain cells of the sporogenous tissue scattered through the mass, are destroyed without forming spores, as is also the case in *Equisetum* and in *Tmesipteris.*

THEORETICAL TREATMENT OF RESULTS FROM PSILOTACEÆ.

The observations above detailed show that the study of development by means of sections supports the observations of SOLMS, rather than those of JURANIYI, GOEBEL, and BERTRAND. We conclude from examination of sections, very clearly in the case of *Tmesipteris,* though with less clear demonstration in the more complex case of *Psilotum,* that the synangium is a product of the adaxial surface of the sporangiophore, and that it arises immediately below the organic apex of that part. These facts strengthen the conclusion already drawn by SOLMS from external observation, that the whole sporangiophore is a member of foliar nature, and that it is not composed of a shortened axis and leaves, as JURANIYI suggested. I would add that, even if the synangium had proved to be terminal, there would be no need to have recourse to so cumbrous, and I think improbable, an explanation of the fact, for to me it seems just as probable that a synangium may occupy the apex of a leaf, as that a sporangium may be found at the apex of an axis; in this case the terminal or lateral position of the synangium would not affect the argument. This seems to have been the view of PRANTL also.

The lateral position of the synangium being demonstrated, and the conclusion arrived at that the whole sporangiophore is a foliar structure, it remains to draw comparisons with other plants, and in the first place with those to which the affinity of the Psilotaceæ has always been recognized, viz., the Lycopodiaceæ. The comparison will first relate to *Tmesipteris,* which is probably nearer akin to *Lycopodium* than is *Psilotum.* If figs. 120, 121, 124 of *Tmesipteris* be compared with fig. 58 of *L. clavatum,* or better with figs. 71–74 of *L. alpinum,* the similarity will be obvious; in both cases an outgrowth has appeared on the upper surface of a leaf, involving a number of cells, which are undergoing anticlinal and periclinal divisions; but whereas the growth in

Lycopodium is close to the base of the sporophyll, that in *Tmesipteris* is strongest close to the apex; this is the most salient point of difference at this stage. It may, however, be noted that an intermediate condition is seen in *Isoetes* (compare figs. 105, 106, 107), where the sporangium is seated slightly above the basal limit of the sporophyll; also in *Lepidostrobus*, where also there is an interval between the lower limit of the sporangium and the insertion of the sporophyll on the axis; also that there is some variety in the relation of the sporangium to the sporophyll in *Selaginella* and *Lycopodium*.

The tangential section of the sporangium in certain species of *Lycopodium* shows a very considerable curved series of archesporial cells (figs. 42, 64, 76); in *Tmesipteris*, the cells so exposed appear to be only two (figs. 127, 129, 130); this difference is, however, not very material, for we find somewhat similar variation within the genera *Lycopodium* and *Selaginella*. Thus, while the number of archesporial cells seen in tangential section in *L. clavatum* is large, in *L. phlegmaria* (fig. 50) it is at most four, or perhaps even two; accordingly, this difference is one of only secondary importance. Some of the various ways in which the dimensions of Lycopodinous sporangia may vary have been discussed above (p. 536, &c.); it has been shown that there is variety within the genus *Lycopodium* in the number of archesporial cells as seen in the radial section, *L. Selago* showing the simplest type, with only one archesporial cell thus seen (fig. 36), while *L. clavatum* will serve as a more complex type with three (fig. 58). It has been pointed out (p. 532) that in *Isoetes* the number of archesporial cells exposed in the radial section is larger (figs. 105, 106), while the existence of this more extensive sheet of archesporial tissue is accompanied by the formation of trabeculæ, which are, as regards their origin, sterilized portions of a potential sporogenous tissue. It has also been above suggested that the large oblong sporangium of *Lepidostrobus* owes its origin to a similar extensive development of the sporogenous tissue, which probably stretched in a radial direction for a considerable distance along the upper surface of the sporophyll (fig. 94); though not septate, the cavity of the sporangium is traversed by trabeculæ (figs. 96–103), or even irregular continuous plates (figs. 132, 133) of sterile tissue; at present, unfortunately, no developmental data are available for proving whether or not these sterile tissues were derived from a potential archesporium, but their great similarity to the sterile trabeculæ of *Isoetes* makes it probable that they were.

Like *Lycopodium* and *Isoetes*, *Tmesipteris* shows also an extensive potential archesporium, but here it occupies a narrow zone on the upper surface of the sporangiophore, and extends for a considerable distance in a radial direction, stretching to its extreme apex. It is not, however, fertile throughout in normal synangia, for while the portions at either end develop so as to supply the contents of the two large loculi, the middle portion is sterile and develops normally as the septum. Thus, on developmental grounds, it appears that the synangium of *Tmesipteris* corresponds in position to the sporangium of *Isoetes* or *Lepidostrobus*, being produced on the upper

surface of the sporophyll or sporangiophore, but at some little distance from the base, and differing in this respect only in a minor degree from other Lycopods; this points towards the homology of the sporangium of the Lycopods and the synangium of *Tmesipteris*, that is, the homology of a single sporangium with a synangium bearing two loculi—a non-septate with a septate body. Clearly, before such a conclusion could be admitted, it will be necessary to examine the evidence very closely.

There are three lines of evidence—(i) from comparative anatomy of the plants in question; (ii) from comparative study of normal development and mature structure of their spore-bearing organs; (iii) from study of abnormalities.

(i) I have already shown elsewhere ('Annals of Botany,' vol. 7, p. 329) that the vascular tissues of *Lepidostrobus Brownii* show an extraordinary similarity to the corresponding tissues of the *Psilotaceæ*; it may be stated that this correspondence is closer than with any other living plants, closer even than with *Lycopodium*. The affinity of *Lepidodendron* has always been recognized with the Lycopods; we now see that, as regards the vascular system, it is more especially with that outlying group of Lycopodineæ, viz., the Psilotaceæ.

(ii) The evidence under our second head in support of the homology has been dealt with at length in the preceding pages, and need only be again summarized here as follows:—(*a*) the position of the synangium of *Tmesipteris*, and its origin from the upper surface of the sporangiophore (sporophyll), is similar to that of *Lepidostrobus* or *Isoetes*; (*b*) in the general form of the synangium and in the structure of the wall, the synangium of *Tmesipteris* resembles the sporangium of *Lepidostrobus Brownii* (compare (figs. 93 and 146); (*c*) the function of the two parts is the same; (*d*) in *Isoetes*, and also in *Lepidostrobus*, sterile trabeculæ, or plates of tissue, run up into the sporogenous cavity corresponding in this respect, though not in detail of outline, to the septum of *Tmesipteris*; (*e*) the origin of the tissue of the septum in relation to the sporogenous tissue is similar in *Tmesipteris* to the origin of the trabeculæ in *Isoetes*. Its origin in *Lepidostrobus* has not been observed, but there is reason to believe that there was similarity in this respect between these two plants.

(iii) The most interesting evidence is that derived from abnormal synangia of *Tmesipteris*, which show that (*a*) where the form is simpler, and resembles more closely that of the sporangium of *Lepidostrobus, the septum may be partially or completely abortive, and the synangium be thus replaced by a simple sporangium*; (*b*) it has been demonstrated that in such cases *the tissue which would normally form the septum may develop as tapetum, or even as sporogenous tissue.* It is thus seen that there is no essential difference between the tissue which may be formative of septum, and that which may be sporogenous, since the one can pass on occasions into the other. It will be noted that this observation is the converse of what Professor GOEBEL has demonstrated in *Isoetes*; he there showed that *part of a potential archesporium may be sterilized, and develop as trabeculæ.* I have now shown the converse, viz., that *tissue, which, though similar in origin to the sporogenous tissue, usually forms a*

septum, may on occasions form spores. The conclusion is that, in dealing with such cases, the presence or absence of trabeculæ or a septum is a phenomenon of secondary moment from the point of view of homology, and, in fact, *there is no essential obstacle to recognizing homologies, between parts of which one may be partially or completely septate, the other non-septate.*[*]

From the facts and the reasoning thus briefly stated, I conclude that *the synangium of Tmesipteris is homologous with the sporangium of other Lycopodineæ*, the nearest correspondence being with *Lepidostrobus* and *Isoetes.* It may be looked upon as an amendment on the type of sporangium in these plants; their trabeculæ, though irregular, doubtless yielded physiological, and possibly also mechanical, support. A comparatively slight rearrangement and consolidation of the trabecular tissue of *Isoetes* or of *Lepidostrobus* might result in the complete partition of the sporangium into two loculi, as it is seen normally in *Tmesipteris;* or the converse view is possible, viz., that the imperfectly septate sporangia of the type shown in *Lepidostrobus* or *Isoetes* may have resulted from reduction of completely septate types. This question will be discussed later. At present it may be stated that the former view seems the more probable.

The case of *Psilotum* appears at first sight more difficult; there can, however, be no doubt that the synangium of *Psilotum* is homologous with that of *Tmesipteris*— the similarity of position and structure, and the occurrence of trilocular synangia in *Tmesipteris* (fig. 156), are sufficient evidence of this. Other observers have noted variations from the normal trilocular synangium in *Psilotum.* A reduction of loculi to two is not uncommon, though it appears to be frequently the result of mere arrest of one of the loculi. In other cases the number of loculi may be increased to four or five (SOLMS, *loc. cit.*, p. 174). From such observations it follows that *the number of loculi need not affect our view of homology;* whether the number of loculi be one (fig. 160), two (fig. 154), three (fig. 156), or four, or five, the synangium of the Psilotaceæ is still homologous throughout, while, as above concluded, it is also homologous with the sporangium of other Lycopodineæ.

The presence of the two leaf-lobes, in the fertile leaves of these two genera, is doubtless one of their most peculiar features, as well as most difficult to harmonize with the condition of the leaf in allied plants. The Lycopodineæ are so strongly characterized by the simple form of their leaves, that these parts of the Psilotaceæ call for special attention. It has been repeatedly remarked that the leaves of *Psilotum* and *Tmesipteris* may appear double, though no synangium may be attached, but these cases probably arise by abortion of a potential synangium, and are not to be looked upon as double leaves normally produced; in fact the vegetative leaf in these plants is normally simple, while the fertile sporangiophore bears lateral lobes;

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[*] A somewhat similar case is to be found in the genus *Najas,* in which the single anther may be quadrilocular in *N. major,* but only unilocular in *N. minor.* EICHLER, 'Blüthendiagramme,' p. 82.

these facts must be accepted as they stand, though the Lycopodinous leaf is else-where simple, and though it is decidedly exceptional to find sporophylls more elaborate in form than foliage leaves of the same plant. It must not be forgotten that the origin of the sporangiophore is like that of the foliage leaf, and that in *Tmesipteris* the distinction can only be drawn when the development has proceeded to a considerable extent, while the lateral lobes make their appearance *later* than the synangium in both genera, and the synangium appears below the apex of the sporangiophore. These are the facts on which the foliar view is based, notwith-standing the peculiarity of the lobed leaf when mature.

But, though peculiar, the lobes of the fertile leaves may be seen to serve a useful end ; here, as elsewhere, the protection of the young synangia is a matter of importance ; it is obvious that two lateral leaf-lobes would offer more protection than a single terminal growth of similar form and size ; the result is shown for *Psilotum*, in figs. 139, 140, and for *Tmesipteris*, in fig. 145, where the synangium is closely invested by the protecting lobes. Moreover, it is to be remarked that in such cases as these, where the synangia appear as very considerable and bulky bodies on the adaxial face of the leaf, the foliage leaf itself being also flattened in a median plane, the presence of an extended leaf-apex would be highly inconvenient in the packing of the parts in the bud, for the result would be a lax bud, in which the individual parts would be greatly exposed. By the arrest of the apex of the leaf, and substitution of the lateral lobes, as implied in the hypothesis above put forward, a more compact bud is produced, and the synangia are better protected. The arrest of the apex of the leaf may, perhaps, be compared with a similar arrest in the flower of certain Angiosperms, where mechanical inconvenience has led to abortion of stamens or carpels.

On the ground of the above facts and considerations, I conclude that the shoot of the Psilotaceæ (apart from branching in the ordinary sense of the word, which is much more common in *Psilotum* than in *Tmesipteris*) is a simple shoot, consisting of an axis, bearing sporangiophores and foliage leaves. These alternate with one another in irregular zones, and in *Tmesipteris* the transition from foliage leaves to sporangiophores may be frequently repeated, in *Psilotum* the repetition is less frequent. The condition is thus similar to that in *Lycopodium Selago* with its successive sterile and fertile zones. The abortive synangia correspond to the abortive sporangia in this and other species of *Lycopodium*, and accordingly the general considerations as regards origin from a simple strobilus, which were laid down, on p. 535, for the genus *Lycopodium*, will apply with equal force to *Psilotum* or *Tmesipteris*.

CONCLUDING REMARKS.

In the above pages I have described the results of an examination of the spore-producing members of the strobiloid Vascular Cryptogams, viz., Equisetineæ, and

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Lycopodineæ, including *Lepidodendron, Isoetes,* and the Psilotaceæ. It may here be remarked that the prime object which I have kept before me has not been directly to trace "homologies" between the parts of various Vascular Cryptogams. It is quite an open question whether true phylogenetic homologies are really to be found between parts of plants which are so distant from one another systematically as, for instance, *Equisetum* and *Lycopodium;* but when plants appear to belong to the same natural series, as, for instance, *Lycopodium, Selaginella, Lepidodendron, Isoetes, Tmesipteris,* and *Psilotum,* then I think that homologies may fairly be traced between their parts. This has not, however, been the first object : the chief end in view has been to disclose some main features of the manner in which the advance from some Algal-Bryophytic ancestry to the Vascular Cryptogams may have been brought about. On earlier pages (p. 485) this question has been first approached by comparative study of the Bryophyta, especially the Hepaticæ. The conclusions arrived at by LEITGEB, the greatest specialist on the minute study of the Hepaticæ, as to the probable mode of progression from the simpler to the more complex types of these plants, suggested the idea that traces of somewhat similar lines of advance might be found among living Vascular Cryptogams ; and that these might throw some light upon the question of transition from the Bryophytes to the more complex vascular plants. Four questions relating to this subject were put forward (p. 494), and we shall now see how far answers may be found to them from the observations and comparisons above described.

(1.) Are sterile cells distributed among the sporogenous cells in any Vascular Cryptogams ?

In answer to this we find sterile cells in large numbers distributed through the sporogenous masses in *Equisetum, Tmesipteris,* and *Psilotum.* The criticism may be advanced that this is merely a physiological phenomenon depending on nutrition ; but in this I see no real objection, for such physiological phenomena have largely affected the course of evolution, and, in fact, morphology should be looked upon as little more than the study of stereotyped physiology. In view of the facts ascertained from the Hepaticæ, we cannot pass over the existence of these sterile cells as a matter of no morphological importance. It is true they do not form firm walls, or develop into cells such as the elaters : they are to be compared rather with the sterile cells in the sporogonium of *Oxymitra* (LEITGEB, *loc. cit.,* Heft 4, p. 43). But whatever comparisons be drawn, the fact remains that sterile cells in large numbers are present in the sporangia of the plants above named.[*]

(2.) Do any Vascular Cryptogams show distinct parts which may be correlated in position, structure, development, and function with the sporogonial head of Bryophyta ?

The strobilus of *Equisetum* is in function the counterpart of the sporogonial head : its normally terminal position is also similar, though the shoot on which it is borne is more complex in structure and in branching. In early stages of

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[*] Similar sterile cells are also found in *Ophioglossum.*

development it is not unlike certain sporogonial heads, but as development proceeds it becomes much more complex, showing first outgrowing sporangiophores, and subsequently separate sporangia. While still young, it is possible to recognize superficial cells which will give rise to the essential parts of the sporangia, at a time when the sporangiophores are only slightly projecting beyond the general surface of the strobilus. Like most sporogonial heads it is usually sharply limited below, and rarely shows the power of branching. Similarly, with *Phylloglossum*, the strobilus holds a position comparable to the sporogonial head, though more complex in structure. Its apical growth, while young, is not unlike that of some sporogonia. In most cases it is sharply limited below, and is rarely branched. But in *Lycopodium* and *Selaginella* the strobilus is not so limited, and, as above explained, there is good reason to believe that the chief vegetative region of these plants is the result of sterilization of the lower parts of an extended and branched strobilus. Such development would attain its highest complexity in the tree-like trunks and branches of *Lepidodendron*, of which the cone (*Lepidostrobus*) would still represent the fertile portion of the sporogonial head. In the *Psilotaceæ* the strobili are more lax: as above pointed out, however, on the grounds of development and histology, as well as general comparison of form, we conclude that the case is similar to that of the *Selago* type of *Lycopodium*. Lastly, *Isoetes* may be regarded as a simple strobilus of which certain sterile leaves intervene between the fertile ones. In fact, all the stroboloid types hitherto examined, will fall in, without undue straining of the idea, with the working hypothesis, that the strobilus is the counterpart of a sporogonial head : in function and position the correspondence is usually plain enough : the structure, external form, and details of development are, however, decidedly more complex in the vascular plants, and the correspondence is apparently less close in proportion as the appendicular organs are of relatively larger size.

(3.) Are the sporangia borne in such relation to one another as to suggest a common origin by subdivision of simpler parts ?

In *Phylloglossum, Lycopodium, Selaginella, Lepidostrobus,* and *Isoetes* the sporangia are isolated, a single sporangium being borne on each sporophyll ; in these genera there is in the arrangement of the sporangia no obvious suggestion of their having been the result of subdivision of simpler parts. But in *Equisetum* and in the Psilotaceæ the case is different. Each sporangiophore of *Equisetum* bears a number of sporangia ; in the young state these appear sunk in the margin of the slightly protuberant sporangiophore ; but when mature they project as apparently more distinct parts. The younger state may be taken as an indication of the probable mode of origin by descent, in which case the view would be arrived at that the sporangia of each sporangiophore were originally more closely associated together, and it will not appear improbable that they may have had a common origin, the tissue now intervening between them having been completely sterilized. But before such a suggestion can be seriously considered exact evidence should be forthcoming

that sterilization can take place; this will be found in the answer to question (4). In the Psilotaceæ the sporangia are grouped in synangia, the number of loculi varying from one to five; their appearance and arrangement certainly would suggest the idea of a common origin, by subdivision of simpler parts. The similarity of the synangium of *Psilotum* to the sporangiophore of *Equisetum* would indicate that the mode of origin of these parts was probably somewhat similar.

(4.) Is there direct evidence in any Vascular Cryptogam of conversion of potential sporogenous tissue into masses of sterile tissue, or conversely of conversion of sterile septa into spore-producing cells?

Professor GOEBEL has shown that the trabeculæ of *Isoetes* are derived from a potential archesporium; this I can endorse, and it would, therefore, appear that the trabeculæ are parts of a potential sporogenous tissue converted into sterile tissue; the same appears to be not improbable for *Lepidostrobus*.* GOEBEL remarks ('Annals of Botany,' 1892, p. 358) that he "would only attach a biological, that is, an adaptive, significance to the fact." My point is that the requirements so well met by *Isoetes* in the formation of its sterile trabeculæ, probably made themselves felt in other plants with large spore-bearing members, and the adaptations to meet them, if successful, would become stereotyped as permanent morphological facts. In *Tmesipteris* also, the normal septum is similar in origin to the sporogenous masses, and is undistinguishable from them while young; thus, from the developmental point of view it may also be looked upon as a result of sterilization. But, conversely, it has been shown that in certain abnormal synangia of *Tmesipteris* the septa may be completely absent, while in others it has been demonstrated that those tissues which normally form the septum may become sporogenous; there is accordingly no absolute and essential difference between tissue of the septum and the sporogenous tissue in the septate synangium of *Tmesipteris*.

Thus, our observations afford answers to the four questions above put forward; but it is true that the evidence is neither over-abundant nor conclusive. Those who best appreciate the greatness of the gulf between the Bryophyta and the Vascular Cryptogams would least expect it to be abundant. Its value is to be estimated in connection with that derived from the Bryophyta; the quotations above given from LEITGEB (p. 487, &c.) show that he deemed that the progress of sterilization in certain series of Liverworts was demonstrated, while comparison leads to the probability of further formation of sterile tissues in such plants as the Anthoceroteæ, in which elater-like cells, similar to those which appear to have undergone consolidation to form the columella, are dispersed through the sporogenous tissue. Somewhat similar evidence, though not so cogent, leads to the conclusion that sterilization, resulting in

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* The same is also the case in *Frullania*, where "elaters" of trabecular character, and attached at both ends, are formed from sister cells of those which, after division, give rise to the spores. See HOFMEISTER, 'Higher Cryptogamia' (Plate 12, fig. 9), and LECLERC DU SABLON, 'Ann. Sci. Nat.,' Bot., 7 série, vol. 2, 1885, p. 130, Plate 7.

sterile tissue-masses or isolated sterile cells, has taken place also among the Vascular Cryptogams. Such evidence is of the greatest importance in connection with our hypothesis of the origin of the strobilus from a sporogonial head. While I know of no facts which present a real obstacle to the acceptance of the hypothesis, a large number of facts relating to external form, internal structure, and development, which have been discussed in previous pages, fall in with the theory. In the absence of more extensive and more direct evidence, therefore, the whole question before us must (like so many problems relating to descent) resolve itself in great measure into a balance of probabilities. In view of the facts as a whole, it seems to me at present probable that the strobilus of the *Equisetineæ* and *Lycopodineæ* is the counterpart of a sporogonial head, such as that seen in the Bryophyta ; that it attained its present condition by partial sterilization of the originally continuous archesporium, and outgrowth of the isolated parts, together with external protective tissues, as sporangia, while there were also formed, probably simultaneously, appendicular organs (sporangiophores and sporophylls), which bear them. That in certain cases (Psilotaceæ and probably Equisetineæ) the original sporangia underwent a further subdivision, by formation of sterile septa, the result being synangia of various types. *Isoetes*, and probably *Lepidodendron*, are to be taken as examples of such sterilization resulting in trabeculæ in place of complete septa. The sterilization thus involved would be similar in kind to that which was so ably traced by LEITGEB in the Liverworts. A point of special interest in connection with this is the presence of sterile cells in the young sporangia of *Equisetum* and the Psilotaceæ, scattered through the sporogenous masses ; similar cells have been observed by ROSTOWZEW ('Recherches sur l'Ophioglossum vulgatum, L.,' Kjöbenhavn, 1891, p. 28, Plate 2, fig. 5) in the Adder's-tongue, and I have been able to confirm the fact. It thus appears that in *Equisetum*, *Psilotum*, *Tmesipteris*, and *Ophioglossum*, all types in which the sporangia are closely associated together so as to form synangia, a considerable proportion of the potential sporogenous tissue is sterile, the cells yielding up their substance to nourish their fellows, which develop as mature spores. This cannot be looked upon as a mere coincidence ; it demands careful consideration, both from the physiological and morphological aspects.

From the physiological point of view the sterile cells act the part of a diffused tapetum ; they become disorganized, and the substances which compose them are taken up into the developing spores. Developmentally they are sister cells with those which produce spores,—they are, in fact, potentially sporogenous cells, and the question may be asked, why they do not develop as such. It would appear that want of nutrition determines the matter, and I think the state of the case is probably as follows : these plants have increased the bulk of their potential sporogenous tissue beyond the point at which it can be wholly matured, and the result is an arrangement by which the number of spores actually produced can be advanced to the largest figure which the plant will be able to ripen. In *Psilotum* it has been

distinctly seen that certain cells of the sporogenous mass first assume denser proto-plasmic contents, and that the number of these increases up to a certain limit when the sporogenous cells separate from one another ; it is probably the condition of the plant at the time as regards nutritive supply which will determine this limit, a larger proportion becoming sporogenous in a well-nourished plant, and a smaller number in an impoverished plant. In this connection, it may be noted that though my specimens of *Psilotum* came from the Glasgow garden, those of *Tmesipteris* were grown in their native country, and both showed the potential sporogenous tissue only partially fertile.

Examining the peculiarity, which these plants share, from the comparative and morphological side, it may be noted that in allied forms, such as *Phylloglossum*, *Lycopodium*, *Selaginella*, *Isoetes*, and in most Filicineæ other than *Ophioglossum*, the large majority, or even all of the cells of the sporogenous mass form spores ; a few odd cells may suffer arrest and disorganization, as has been seen in the micro-sporangia of *Selaginella* and *Isoetes*, but (with the exception of the megasporangia of the latter genera) the arrest and destruction of a considerable proportion of potentially sporogenous cells is not a distinct feature ; this makes the matter still more deserving of consideration.

It has been noted that in the *Anthoceroteæ* LEITGEB saw evidence of sterilization of tissues, resulting in the formation of the central columella ; in addition to this there are also present in *Anthoceros* sterile elaters which originate with the sporo-genous cells from the archesporium ; thus *Anthoceros* shows both the result of a more general sterilization as tissue masses, and individual sterilization of cells scattered through the potential sporogenous tissue. Now in *Equisetum*, *Psilotum*, and *Tmesipteris* we see plants with sporangia grouped in such a way as to suggest, on our general hypothesis, an origin by subdivision of simpler sporangia, the septa being a result of partial sterilization ; but in addition to this (as also in *Anthoceros*) sterile cells are scattered through the potential sporogenous masses ; it is true these cells, in the vascular plants quoted, do not form cell-walls as do the elaters of *Anthoceros*, but morphologically speaking, in both cases we have to deal with sterilized cells of a potential sporogenous mass, and in both cases the organisms concerned are such as give good reason to recognize in the presence of masses of vegetative tissue (columella and septa), the results of previous sterilization. In the presence of these cells scattered through the sporogenous tissue, I should be disposed to recognize that *Equisetum*, *Psilotum*, and *Tmesipteris* show a condition which, with little further modification, might lead to further subdivision of their sporangia ; this would be readily effected by the consolidation of such scattered cells into coherent masses, in a manner illustrated by *Metzgeria*, *Aneura*, and the Anthoceroteæ.

It has been noted that *Ophioglossum* shows a similar condition : this interesting fact will be considered in due course when the Filicineæ are under discussion.

If the reasoning brought forward in the above pages be accepted, the strobiloid

Vascular Cryptogams would take a place in the *ascending* series of vascular plants. This was clearly the opinion of NAEGELI. But certain other writers have looked upon them as examples of reduction : thus STRASBURGER ('Bot. Zeit.,' 1873, No. 6, &c.) contemplated it as probable that the sporangia of *Lycopodium* and *Selaginella* were the result of contraction of the whole fertile frond of the Ophioglossaceæ, while the synangium of *Psilotum* represented for him the result of reduction of a whole strobilus of *Lycopodium*. Those also who look upon the Leptosporangiate Ferns as the nearest of living vascular plants to the Bryophyta are often disposed to regard the strobiloid Pteridophyta as comparatively reduced types : and doubtless some will see in the sterile cells scattered through the sporogenous masses of *Equisetum*, *Psilotum*, and *Tmesipteris* evidence of such reduction. It is true the Pteridophyta at present living are smaller than many of the individuals of former ages : but their sporangia do not show evidence of reduction of type, or of coalescence, as compared with their fossil relatives. If the living strobiloid types have been reduced as regards their spore-bearing parts, their relatives, the Calamarieæ and Lepidodendra, must also have been reduced ; that is, some of the earliest fossil plants of which we have any accurate knowledge would have to be looked upon as reduced types, and in the descending scale of evolution. This is doubtless possible, but seems to me by no means probable ; in fact, it is very much like a *reductio ad absurdum*. Accordingly I conclude on this, as also on grounds of general comparison, that the more probable view is that the strobiloid types, both ancient and modern, are in the ascending scale of evolution, as they must certainly be if our working hypothesis is sound.

I have now examined types of all the genera of living Vascular Cryptogams, exclusive of the Filicineæ. On the latter, rather extensive observations have also been made, but I propose to hold back such results as are already in hand till my investigations of the Filicineæ have been completed. In the meanwhile it may be remarked that the main lines of investigation and of argument pursued in the above pages will be found applicable also to the Filicineæ.

I do not propose to discuss the results from a more general or from a phylogenetic point of view till the close of the remaining part of this Memoir, which will have as its subject the morphology of the spore-bearing members of the Filicineæ.

In conclusion it may be remarked that there appears to be good reason, both from grounds of comparison and from detailed observation, to believe that one at least of the three possible modes of increase in number of spore-producing masses (separate archesporia) suggested in the introductory paragraphs (p. 484), has played a part in the evolution of Vascular Cryptogams, viz., subdivision and partitioning of an originally simple sporogenous mass, by its partial sterilization and formation of septa. It is not contended that the point has been actually demonstrated, but the facts derived from the study of *Tmesipteris*, when compared with those relating to other Lycopods, and *Isoetes*, make this conclusion appear a probable one. It may remain an open question how important a part this factor may actually have taken in the

evolution of vascular plants : my own opinion is that subdivision has largely, though not exclusively, been the means of increase in number of separate archesporia. But at least I think that both the general considerations and the detailed facts above discussed go far to show that progressive sterilization and partitioning of spore-bearing members are factors which will have to be taken into account in solving the problems of origin and progress of vascular plants.

<div align="center">DESCRIPTION OF FIGURES.</div>

<div align="center">PLATE 42.</div>

<div align="center">*Equisetum arvense*, L.</div>

Fig. 1. Radial longitudinal section of part of a young strobilus, showing two sporangiophores in a very young state. (× 300.)

Fig. 2. Part of an older sporangiophore in radial section, with young sporangium : the group of cells shaded corresponds to the cell shaded in fig. 1. (× 300.)

Fig. 3. Ditto, showing the first periclinal division in the outer cell. (× 300.)

Fig. 4. Ditto; considerably older, and showing cells (×) which are added to the archesporium as the result of subdivision of the outer of the two original cells. (× 300.)

Fig. 5. Ditto; older. Cells marked (×) correspond to those in the previous figure. (× 300.)

Fig. 6. Ditto; a good deal older. All the essential parts of the sporangium are here initiated. (× 300.)

Fig. 7. Oblique section through a sporangiophore of age corresponding to fig. 4, so as to pass through the axis of the young sporangium in a plane at right angles to that of fig. 4. (× 300.)

Fig. 8. A section similar to fig. 7. (× 300.)

Fig. 9. Transverse section through a sporangium of age corresponding to that shown in fig. 6. (× 300.) The arrow indicates the side next to the stalk of the sporangiophore.

<div align="center">*Equisetum limosum*, L.</div>

Fig. 10. Part of a tangential section of a strobilus, which traverses the sporangiophores transversely. *sp.* = stalks of sporangiophores. *a, b, c* = three sporangia cut transversely, and showing extreme differences of size and complexity in sporangia side by side. (× 300.)

Figs. 11, 12, 13. Three sporangia from the same strobilus, cut in median longitudinal section, and showing different types of segmentation, together with difference of bulk. ($\times$ 300.)

Fig. 14. Median section of a rather older sporangium, from near the apex of a strobilus. *v.b.* shows where the vascular bundle is beginning to be developed. ($\times$ 300.)

PLATE 43.

Fig. 15. Median longitudinal section of a sporangium at the base of the strobilus, together with the annulus (*a.*). ($\times$ 300.)

Fig. 16. Tangential section of a sporangium of same age as figs. 11–13. ($\times$ 300.) Compare fig. 11.

Figs. 17, 18. Ditto; more complex specimens. ($\times$ 300.)

Fig. 19. Tangential section of a sporangium in a more advanced state. ($\times$ 300.)

Fig. 20. Apex of an older sporangium in radial section. The tapetum (*t*) is now clearly defined. ($\times$ 300.)

Fig. 21. Part of an older sporangium, showing the tapetum (*t.*) still a clearly-defined band, though the individuality of the cells is lost; within this the sporogenous tissue, of which certain cells (*a*) are abortive. ($\times$ 300.)

Phylloglossum Drummondii, KUNZE.

Fig. 22. A plant of *Phylloglossum,* showing tuber-leaves and strobilus; one sporophyll of the latter is at a distance below the rest, intercalary growth having taken place in the axis above it. ($\times$ 3.)

Fig. 23. A plant of *Phylloglossum* grown in the Glasgow Botanic Garden; the strobilus is branched into two unequal parts. ($\times$ 3.)

Fig. 24. Median longitudinal section through the plant represented as fig. 8 in 'Phil. Trans.,' 1885, Plate 71. *a.* = apex; *l.* = leaf (protophyll); *r.* = root ($\times$ 150.)

Fig. 25 (i., ii., iii.) Successive transverse sections of the young leaf (protophyll) of the plant represented as fig. 10 in 'Phil. Trans.,' 1885, Plate 71. ($\times$ 150.)

PLATE 44.

Fig. 26. Apex of a strobilus in median longitudinal section showing an initial cell (*i*), two sporophylls (*l' l''*), the latter just beginning to be developed; in connection with *l'* is a sporangium, of which the archesporium (*a*) consists apparently of one cell. ($\times$ 325.)

4 C 2

Fig. 27. Another section from the same sporangium, showing further segmentations, which may have been present in the section shown in fig. 26, but made invisible by the method of clearing used. (× 325.)

Fig. 28. A slightly older sporangium in radial section. (× 325.)

Fig. 29. An older sporangium, in which periclinal divisions have begun in the cells of the wall of the sporangium. (× 325.)

Fig. 30. Radial section of a sporangium, in which the sporogenous cells are beginning to separate, but the tapetum is not yet formed from the inner layer of the wall. (× 150.)

Fig. 31. Transverse section of a young sporangium. (× 300.)

Fig. 32. Transverse section of a sporangium, of which one-half is shown ; the stage is slightly younger than that of fig 30. (× 300.)

Fig. 33. Tangential section of a sporangium, of which rather more than one half is shown ; the asterisk indicates the middle of the sporangium. (× 325.)

Lycopodium Selago, L.

Fig. 34. Radial section through a sporophyll (*l*) at the base of which a sporangium is beginning to make its appearance as a slight swelling. (× 300.)

Fig. 35. A similar sporangium, in radial section, rather more advanced. (× 300.)

Fig. 36. Ditto, older ; the archesporium is shaded. (× 300.)

Fig. 37. Ditto, a more advanced stage ; showing very regular segmentation. (× 300.)

Fig. 38. Ditto, showing a less regular type of segmentation. (× 300.)

Fig. 39. Ditto ; still less regular. (× 300.)

Fig. 40. Ditto, older ; the tapetum (*t.*) not yet complete. (× 150.)

Fig. 41. Ditto, older ; showing the spore-mother-cells separated from one another, but not yet divided into tetrads. (× 150.)

PLATE 45.

Fig. 42. Tangential section of a young sporangium of *L. Selago;* the cells numbered i., ii., iii., correspond to those similarly numbered in fig. 36 (× 300.)

Fig. 43. Ditto, older. (× 300.)

Fig. 44. A small part of a similar section from a rather older sporangium. (× 300.)

Fig. 45. Young sporangium, seen in superficial view. (× 300.) *st.* = stem ; *l.* = sporophyll.

Fig. 46. A sporangium of almost the same age seen in transverse section ; compare the line in fig. 42 ; the archesporial cells are shaded in both figures, and numbered (ii.). (× 300.)

Fig. 47. Ditto. (× 300.)

Fig. 48. Ditto, older, before the tapetum is defined. (× 300.)

Fig. 49. Ditto ; half of an older sporangium in which the formation of the tapetum (*t*.) is almost complete. (× 300.)

Lycopodium Phlegmaria, L.

Fig. 50. Tangential section of a young sporangium, showing the archesporium, referable to not more than four, and possibly to two cells. (× 300.)

Lycopodium carinatum, DESV.

Fig. 51. Transverse section of a sporangium, so as to traverse the sub-archesporial pad (*s.p.*) ; the two ends of the curved mass of sporogenous tissue are cut through, and are shaded ; this is intended for comparison with a transverse section of the " fertile frond " of *Ophioglossum*. (× 300.)

Lycopodium dichotomum, JACQ.

Fig. 52. Part of the wall of sporangium in section ; the tapetum is shaded. (× 150.)

Fig. 53. The same, showing the point of dehiscence. (× 150.)

Lycopodium inundatum, L.

Fig. 54. Radial section of a young sporangium, showing periclinal division in two distinct cells ; two archesporial cells are shaded. (× 300.)

Fig. 55. A similar section of an older sporangium. (× 300.)

Lycopodium clavatum, L.

Fig. 56. Radial section through a very young sporangium, showing the first periclinal division. (× 300.)

Fig. 57. Ditto, showing periclinal divisions in two distinct cells. (× 300.)

Fig. 58. Ditto, older, showing result of periclinal division in three cells ; the archesporium thus defined is shaded. (× 300.)

Fig. 59. Older sporangium, in radial section, with large sporogenous tissue, so grouped as to be still referable to three original cells ; compare fig. 58. (× 300.)

Fig. 60. Radial section of an older sporangium ; *st.* = the adaxial side : the sporogenous tissue not shaded, it is still referable to the three parent cells, the lower limit of the groups being here unusually irregular. The tapetum is shaded. (× 300.)

Fig. 61. Radial section of a sporangium, which shows early periclinal divisions of the superficial cells; the fate of the inner cells (*o, o*) is uncertain; *v.b.* = vascular bundle. (× 300.)

Fig. 62. Ditto. (× 300.)

Fig. 63. Tangential section of a sporophyll bearing a sporangium of age corresponding to that in fig. 56; the periclinal walls have not yet appeared in all the parent cells of the sporangium. (× 300.)

Fig. 64. A similar section of an older sporangium; in both of these the archesporium is shaded. (× 300.)

Fig. 65. Part of a tangential section of an older sporangium; *t.* = tapetum; *v.b.* = vascular bundle; *s.a.* = sub-archesporial pad; sporogenous tissue is deeply shaded. (× 300.)

Fig. 66. Small part of the sub-archesporial tissue, showing a process rising upwards into the mass of spore-mother-cells. (× 300.)

Fig. 67. A similar part from an almost mature sporangium; the irregular upward processes are now partly disorganized. (× 150.)

Fig. 68. View of the superficial cell-net of a sporangium, as seen from above. (× 300.)

Fig. 69. Transverse section of such a sporangium traversing the archesporium, which is shaded. (× 300.)

Fig. 70. Transverse section of an older sporangium; the three rows of parent cells of the sporogenous tissue may be still recognized. (× 300.)

Lycopodium alpinum, L.

Fig. 71. Radial section through a sporophyll and young sporangium. (× 300.)

Fig. 72. Ditto, the archesporium shaded. (× 300.)

Fig. 73. Ditto, rather older. (× 300.)

Fig. 74. Ditto, the cells marked (×) have apparently been derived by extra periclinal divisions from the superficial cells. (× 300.)

Fig. 75. Radial section of an older sporangium in which the spore-mother-cells are about to separate. The vascular bundle shows a slight extension upwards into the stalk of the sporangium. (× 150.)

PLATE 47.

Fig. 76. Tangential section through a young sporangium, showing an archesporium consisting of twelve cells, which are shaded. (× 300.)

Fig. 77. An upgrowth of the sub-archesporial tissue as a process, which projects between the sporogenous cells. (× 300.)

Selaginella Martensii, Spring.

Fig. 78. Radial section, including apex (*ap.*), and traversing a young sporophyll (*l*)
and sporangium (*x*). (× 550.)

Fig. 79. Ditto, rather older. (× 550.)

Selaginella spinosa, P.B.

Fig. 80. Radial section through a very young sporangium. (× 550.)

Fig. 81. Ditto. (× 550.)

Fig. 82. Ditto. (× 550.)

Fig. 83. Ditto. (× 550.)

Figs. 84–86. Ditto, older. (× 550.)

Fig. 87. Ditto, a good deal older, showing all the essential parts of the sporangium
together with ligule, and part of sporophyll. (× 300.)

Fig. 88. Tangential section of a sporangium of about the same age as figs. 84–86 :
the archesporium is referable apparently to four parent cells. (× 550.)

Figs. 89–90. Transverse sections of sporangia of two different ages. *l.* = ligule,
t. = tapetum, *sp.* = sporogenous tissue. (× 300.)

Fig. 91. Radial section of a megasporangium showing the single tetrad still very
small, and the rest of the potential sporogenous cells arrested. (× 150.)

Fig. 92. *a–w.* Low-power drawings, showing outlines of the sporangia of various
species, in radial, tangential, and transverse sections, together with
parts of the sporophylls : *a, b, c, Phylloglossum Drummondii; d, e, f,
L. Selago; g, h, i, L. phlegmaria; k, l, m, L. inundatum; n, o, p,
L. carinatum; q, r, s, L. alpinum; t, u, v, w, L. clavatum.* (× 18.)

PLATE 48.

Lepidostrobus Brownii, Schpr.

Fig. 93. Wall of sporangium from a tangential section of the cone (No. 7 in the
Museum series of tangential sections) : it shows the outer sclerotic series
of cells (*scl.*), with several layers of thin-walled cells within. (× 150.)

Fig. 94. From a photograph of part of a transverse section of the cone, showing
three sporangia, with the upward projecting sub-archesporial pad as a
median ridge (*r.*). (× 4½.)

Fig. 95. From a photograph of a tangential section of the cone.

Fig. 96. Drawing of a sporangium in tangential section of the cone with its
sporophyll (*sp.*), slightly diagrammatic. *r.* = sub-archesporial ridge ;
v.b. = vascular bundle ; *p* = processes rising from the ridge. (× 8.)

Fig. 97. Part of a radial section of the cone showing a small part of the base of the sporangium ; $r.r.$ = the sub-archesporial ridge, together with the processes $(p.p.)$ which rise from it.   ($\times$ 40.)

Fig. 98. The ridge ($r.$) as seen in tangential section of the cone (compare figs. 95, 96), showing on a larger scale the processes ($p.$) which project far upwards into the mass of spores.   ($\times$ 40.)

Fig. 99. A sporangium from the apex of the cone, cut tangentially (compare fig. 95). The sporangium was not fully matured, and showed very large processes (p) springing from the sub-archesporial ridge, and continuous upwards towards the upper wall of the sporangium.   ($\times$ 40.)

Fig. 100. Another such, with its sporophyll.   ($\times$ 40.)   Figs. 99 and 100 are taken from slide No. 9 of the tangential series in the British Museum.

Lepidostrobus, sp., Hough Hill.

Fig. 101. From a photograph of a *Lepidostrobus* from Hough Hill, supplied by Mr. Lomax, and cut tangentially.   The sporophylls and sporangia are easily seen, while each sporangium shows a dark process rising from the slightly convex sub-archesporial pad, and extending far upwards into the sporogenous mass.   ($\times$ 4½.)

Fig. 102. The base of one of these processes seen under a higher power, and showing the cellular structure of the lower part, though this structure is lost upwards.   ($\times$ 150.)

Lepidodendron Brownii, Schpr.

Fig. 103. Similar section from Schimper's smaller cone, in the British Museum, showing ridge ($r.$) and processes ($p.p.$)   ($\times$ 20.)

PLATE 49.

Isoetes lacustris, L.

$L.$ = ligule ; $v.$ = velum ; $t.$ = tapetum ; $tr.$ = trabeculæ ; $sp.$ = sporogenous tissue ; $v.b.$ = vascular bundle.

Fig. 104. Part of radial section of a plant which has traversed a young leaf in median longitudinal section, the upper (adaxial) surface bears a rather regular layer of cells, as yet not divided periclinally ; these are the parent cells of the sporangium.   ($\times$ 300.)

Fig. 105. These cells are represented in an older state, having divided by both periclinal and anticlinal walls; the inner archesporial cells are shaded. (× 300.)

Fig. 106. A similar section, showing addition of cells (×) resulting from repeated periclinal division of superficial cells to the archesporium. (× 300.)

Figs. 107, 108. Older sporangia with archesporium shaded. (×) = cells believed to have been added by subsequent periclinal division of superficial cells. (× 300.)

Fig. 109. A much older sporangium, already developing as a microsporangium, in similar section, showing the sporogenous tissue as a connected and undifferentiated band. (× 300.)

Fig. 110. Part of an older microsporangium, in similar section, showing the potential archesporium differentiated into trabeculæ (*tr.*) and sporogenous masses (*sp.*), while the tapetum is also clearly defined : at (*) is an extra periclinal division in the wall. (× 150.)

Fig. 111. Transverse section of a sporophyll and microsporangium ; *v.b.* = vascular bundle of sporophyll. Compare tangential sections of sporangia of *Lycopodium* (figs. 33, 43, 64, 65). (× 150.)

Fig. 112. A microsporangium drawn under low power to show the irregularity of the trabeculæ. (× 8.)

Fig. 113. Shows the structure of the trabeculæ, as well as their irregularity ; the superficial layer has developed as a tapetum, which is shaded. (× 150.)

Fig. 114. A microsporangium in longitudinal section. (× 20.)

Fig. 115. The base of one of the trabeculæ of an almost mature microsporangium, still showing cell-structure in its lower part, but disorganized above. (× 150.) Compare fig. 102 of *Lepidostrobus.*

Fig. 116. Transverse section of a sporophyll with a megasporangium, from which the spores have been removed ; the trabeculæ are apparently regular.

Fig. 117, *a* and *b*. A megasporangium which has been so cut as to remove the upper wall (*a*), from which the trabeculæ project into the cavity of the sporangium ; (*b*) is the remainder of the sporangium, *r.* = the ridge, or sub-archesporial pad, which is to be compared with the similar part in *Lepidostrobus* (fig. 94, *r.*) ; it is from this ridge that the irregularly disposed trabeculæ arise. (× 4.)

PLATE 50.

Tmesipteris tannensis, BERNH.

Fig. 118. Median longitudinal section of the apex of a strongly growing stem, showing an initial cell (*x*), but a rather irregular disposition of the segments. (× 150.)

Fig. 119. Apical meristem of the axis of *Tmesipteris* as seen from above: (x) = initial cell, (o) = secondary initials. (× 150.)

Figs. 120 (A and B), 121. Young leaves, in radial section of a bud, showing the way in which they originate on the axis. (× 150.)

Fig. 122. A young leaf as seen in transverse section of the axis. (× 150.)

Fig. 123. A more advanced leaf, probably vegetative; at all events it shows as yet no clear indication of bearing a synangium. (× 150.)

Fig. 124. A very young synangium arising on the adaxial surface of a leaf which is closely similar to fig. 123. In this figure, as also in figs. 120–123, the basal line is more heavily marked. Compare the later figures also. (× 150.)

Fig. 125. Sporophyll bearing a much older synangium; the apical cell (x) may still be seen; the basal line is darkly marked as before, and the sporogenous masses are shaded. (× 150.)

Fig. 125, *bis.* Another specimen of the same, showing very regular disposition of the tissues. (× 150.)

Fig. 126. An older synangium in radial section. (× 150.)

Fig. 127. A vertical section along a line x, x, as shown in fig. 125; l, l are the leaf-lobes. (× 150.)

Figs. 128–130. Transverse sections of sporophylls of successive ages, so cut as to traverse the lower sporangium. l, l = leaf-lobes in fig. 130. (× 150.)

Fig. 131. Transverse section of an older sporangium. (× 150.)

PLATE 51.

Fig. 131, *bis., a–i,* spores from one normal synangium of *Tmesipteris;* a is the usual type; $b–i$ show various abnormal forms, which appear to result from incomplete division of the tetrads.

Lepidostrobus, sp.

Fig. 132. From a photograph of a transverse section of a strobilus, showing part of two sporangia, which have been traversed in a plane above the sub-archesporial pad, but so as to cut through the sterile projections. These take the form of plates, which appear as dark streaks $(st.)$, continuous for a considerable distance in a radial direction through the sporangium. There may be one such streak or plate of sterile tissue, as in the lower sporangium of fig. 132, or two less regular ones, as in the upper sporangium. The section shown in this figure has been slightly oblique: $sp.$ = spores almost mature.

Fig. 133. A similar section showing three sterile plates $(st.)$, which projected upwards into the mass of spores $(sp.)$.

Psilotum triquetrum, Sw.

Fig. 134. Median longitudinal section of a sporophyll. ($\times$ 150.) Compare figs. 124 and 125 of *Tmesipteris*.

Fig. 135. Vertical section of a very young synangium, so as to traverse one of the three loculi. ($\times$ 150.)

Fig. 136. Ditto, older. ($\times$ 150.)

Fig. 137. Ditto, older. ($\times$ 150.)

Fig. 138. Ditto, older, on a lower scale. ($\times$ 100.) The cells shaded are the actual sporogenous cells.

Fig. 139. Transverse section of a synangium, rather older than that in fig. 135. ($\times$ 150.) *l, l* = leaf-lobes.

Fig. 140. Ditto, older. ($\times$ 150.)

Fig. 141. Ditto, one sporangium. ($\times$ 150.)

Fig. 142. Ditto, older. ($\times$ 150.)

Figs. 143, 144 illustrate the disorganization of certain cells of the sporogenous tissue, without forming spores. ($\times$ 150.)

Fig. 145. Transverse section through a sporangiferous bud of *Tmesipteris*. *ax.* = axis, *f.* = foliage leaves, *l.* = lateral lobes, *sy.* = synangia. ($\times$ 20.)

PLATE 52.

Tmesipteris tannensis, BERNH.

Fig. 146. Part of a radial section through a mature synangium, showing the groove between the sporangia, and the insertion of the septum. ($\times$ 150.)

Fig. 147. Radial section through a mature synangium, showing the vascular bundles. ($\times$ 4.)

Fig. 148. Section in the plane of the septum, showing the course of the vascular branches up the margin of the septum. ($\times$ 4.)

Fig. 149. A " double leaf," with abortive synangium (*sy.*). ($\times$ 2.)

Fig. 150. A synangium with the upper loculus abortive. ($\times$ 2.)

Fig. 151. Ditto, with lower loculus abortive. ($\times$ 2.)

Fig. 152. Ditto, with correlative growth, in place of the abortive synangium, inserted on adaxial face. ($\times$ 2.)

Fig. 153. Abnormal sporangiophore (*a*) in lateral, (*b*) in abaxial, (*c*) in adaxial view ; *sy.* probably represents the abortive synangium. ($\times$ 2.)

Fig. 154. A normal sporangiophore from the middle of a fertile zone. ($\times$ 2.)

Fig. 155. A sporangiophore from the limit of a fertile zone, with very small leaf lobes. ($\times$ 2.)

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Fig. 156. A sporangiophore with trilocular synangium. (× 2.)

Fig. 157. Ditto, but less regular. (× 2½.)

Fig. 158. Ditto, with synangium showing no transverse groove. (× 2.)

Fig. 159. Ditto, of type shown in section in fig. 164. (× 3.)

Fig. 160. Ditto, synangium of spherical form, shown in section in fig. 168. (× 3.)

Fig. 161. Median section through a synangium of type shown in fig. 158, with continuous sporogenous mass.

Fig. 162. Detailed drawing from a similar section, showing the tissue, where the septum should normally be present, developing as sporogenous cells (s.) and tapetum (t.). (× 150.)

Fig. 163. Synangium similar to fig. 161, rather more advanced.

Fig. 164. Synangium similar to that of fig. 159 in radial section, showing no septum ; the cavity is filled with sporogenous and tapetal cells.

Fig. 165. Part of these contents drawn in detail from another section of the same series ; the line x, x, shows where the septum would normally be, while a chain of sporogenous cells stretches continuously across it. (× 150.)

Figs. 166, 167. Two transverse sections, the one (fig. 167) higher up, the other (fig. 166) lower down in the same synangium. Fig. 166 shows no septum. Fig. 167 shows a septum cut through, which, therefore, only extended part way downwards into the cavity, from the upper wall of the synangium.

Fig. 168. Transverse section through the spherical synangium shown in fig. 160. No septum is present.

The figs. 132, 133, 161, 163, 164, 166–168 are from photographs kindly prepared for me by Mr. J. REID.

Fig 5

Fig 6

Fig 7

Fig 8.

Fig 10.

Fig 11.

9.
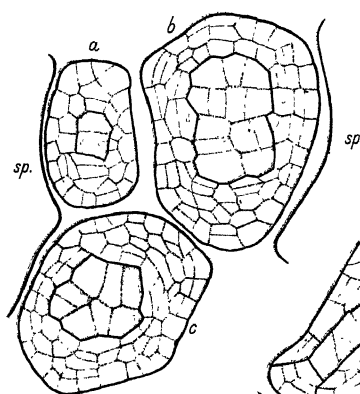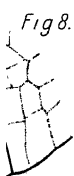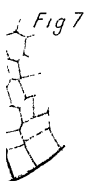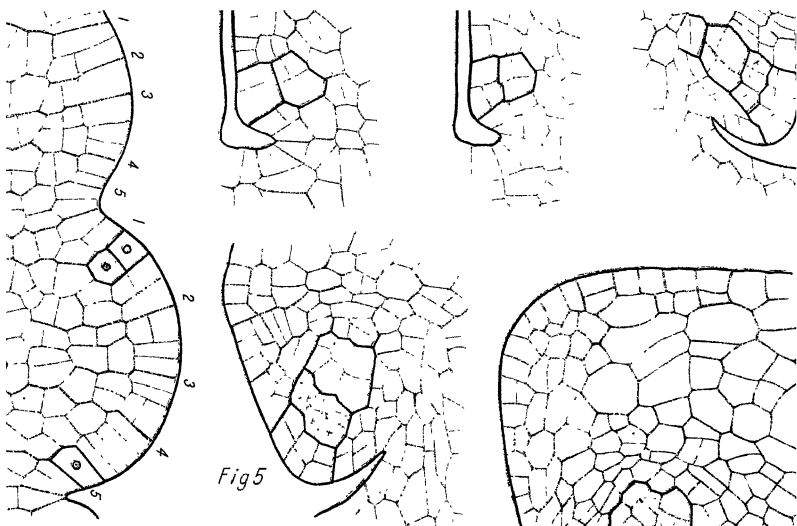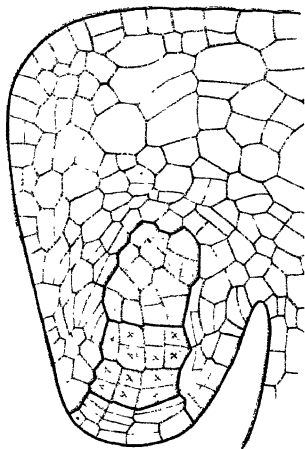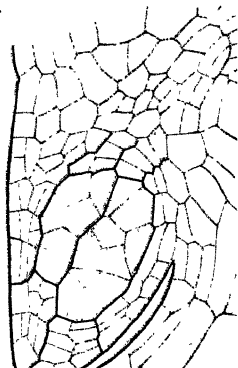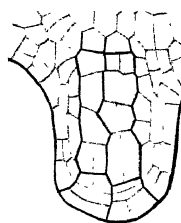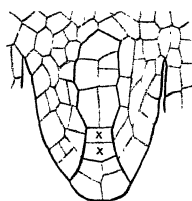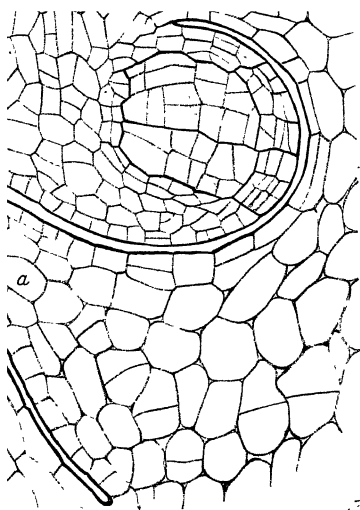
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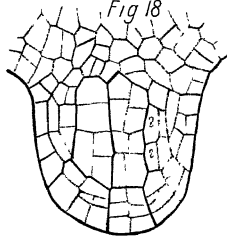
Fig 19

Fig 20.

Fig. 21

Fig 24

Fig. 25.

II.

I.

III.

Fig 22.

Fig 23.

x3

Fig 26.

Fig 30

Fig 31

Fig 35

Fig 33

Fig. 36

37

Fig 41.

Fig 38.
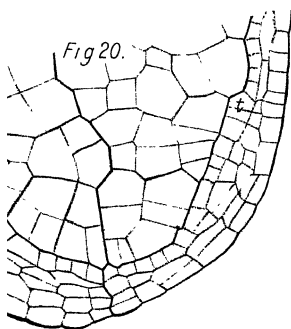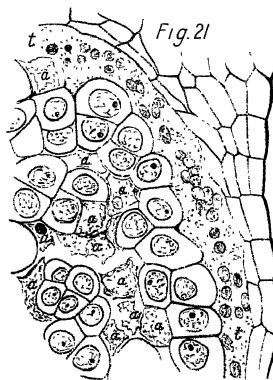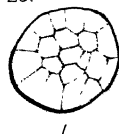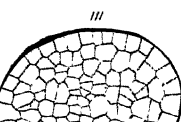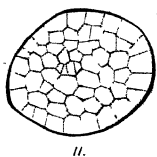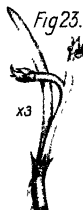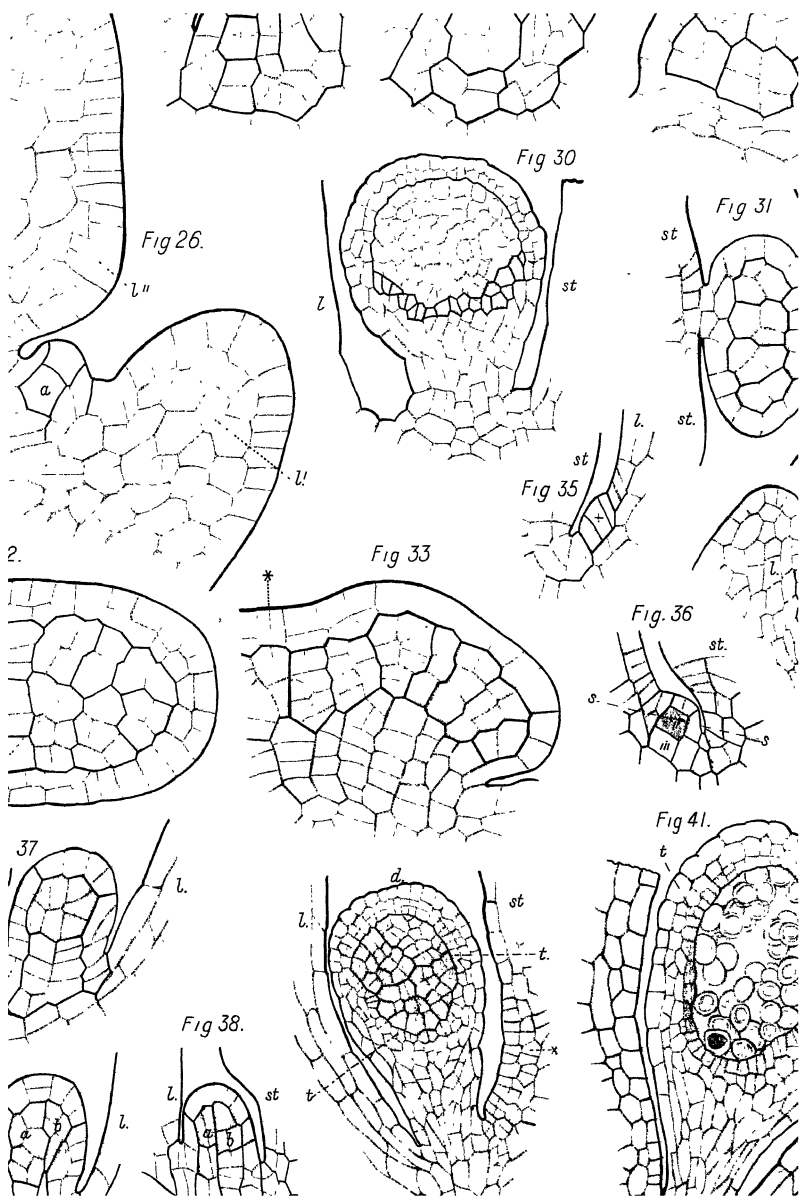
Fig 44.
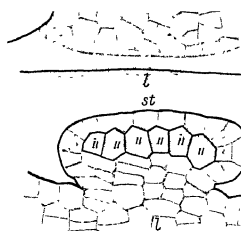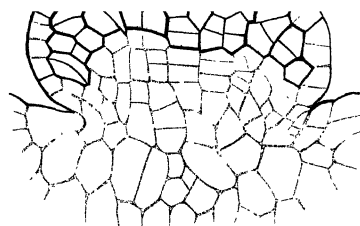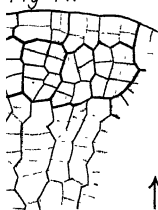
Fig 46

st Fig 48.

Fig 47.

Fig 50.

stem

s.p

leaf
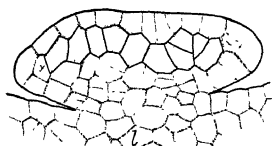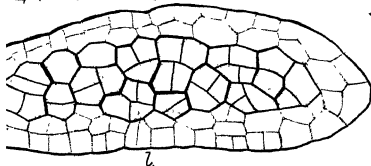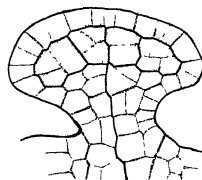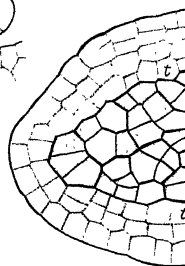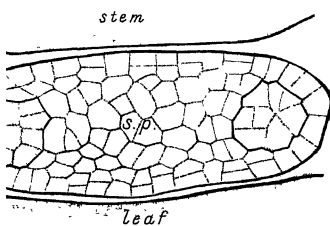
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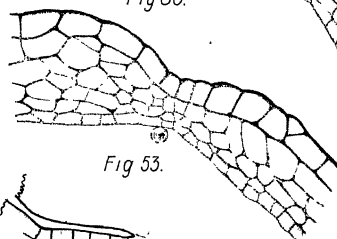
Fig 53.

g 54.

Fig. 56.
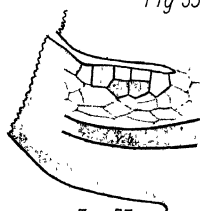
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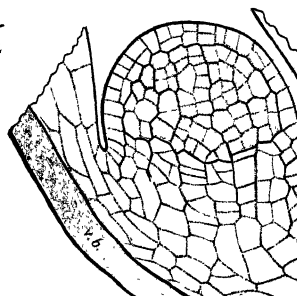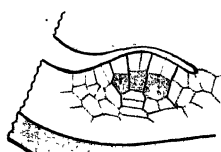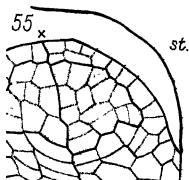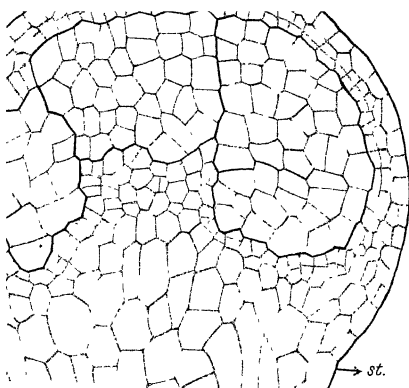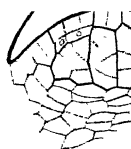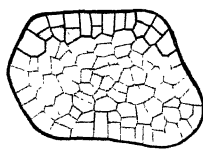
55 ×

st.

Fig 60.

Fig 61.

v b

Fig. 63.
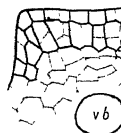
v b
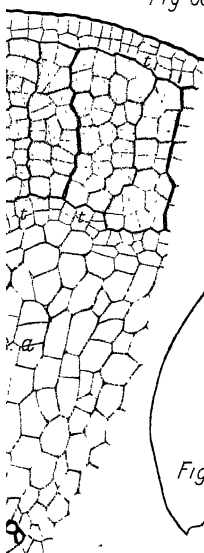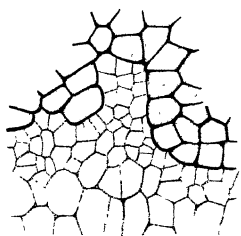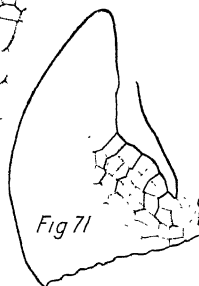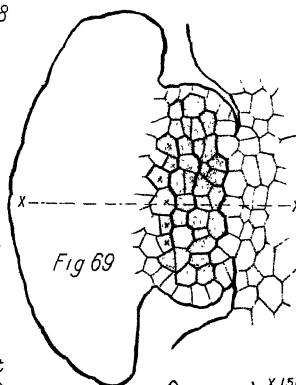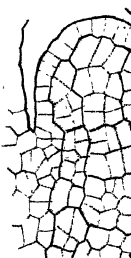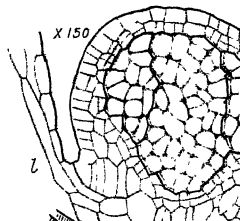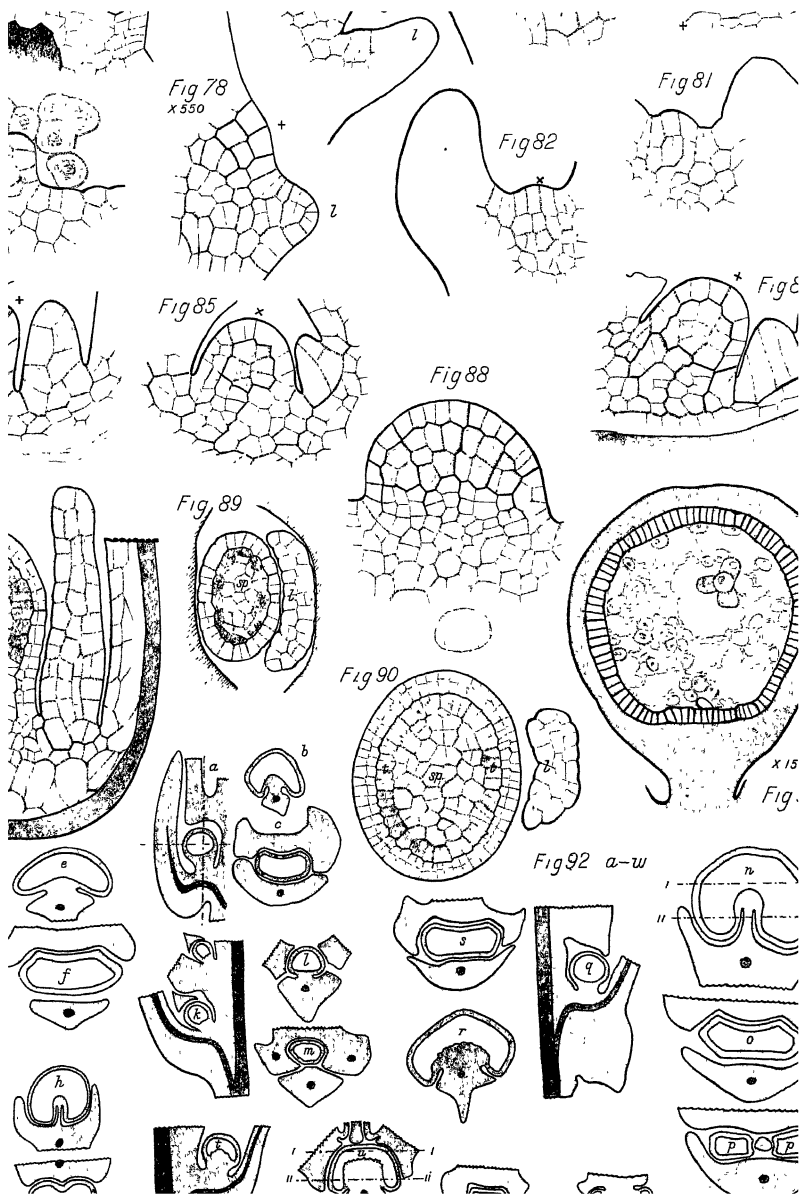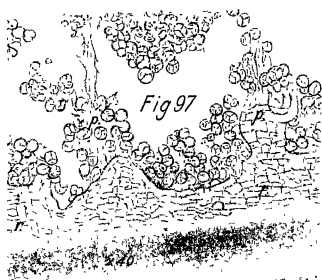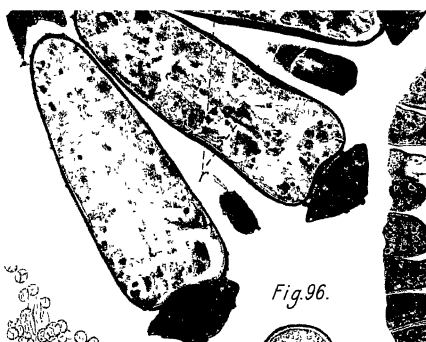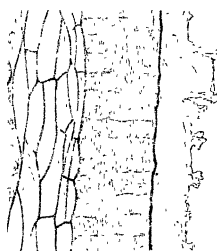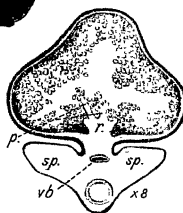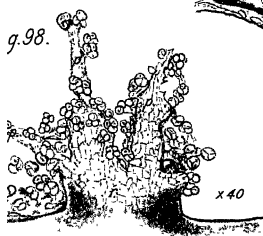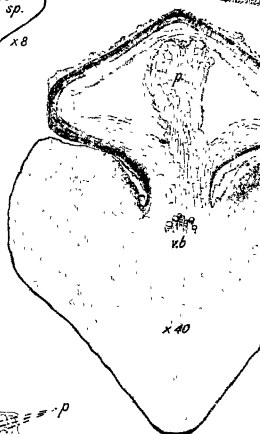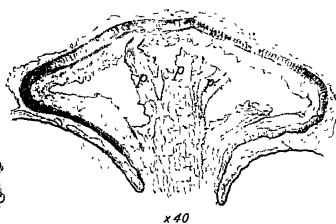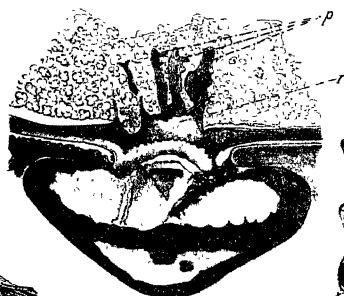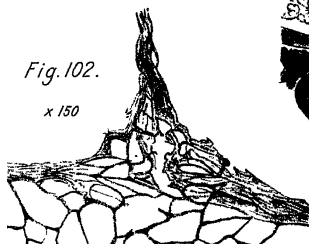
v b

Fig 66

Fig 68

Fig 71

Fig 69

x — — — — x

ig 65

73.

st

Fig 72

X 150

l

Fig 78
×550

Fig 81

Fig 82

Fig 85

Fig 88

Fig, 89

Fig 90

×15

Fig.

Fig 92 a–w

Fig.96.

Fig 97

Fig.99.

p·
p·
T·
sp.   sp.
vb   x8

g.98.

x40

Fig.102.

x150

x40

x40

x20

x40

vb

x40

p

r

Fiq 103.

Fig.107.

Fig.108.

Fig.109.

Fig.110

× 150

Fig.113.

× 150

Fig.112.

tr

× 8

v.b

Fig.114.

v.

× 20

tr.

Fig.115.

tr

Fig.116.

× 8

tr

tr

LIGULE
a

Fig.117.

LIG
b

Fig 122

Fig.124.

Fig.120.

Fig 125

Fig.121.

v.b

p

v.b.

v.b.

F

Fi

Fig.127.

Fig 129.

l

l

Fig.130.

l

l

sp.

st

a *b* *c*
d *e* *f*
g *h* *i*
× 70

st   *w*   *st*

Fig 135

Fig 134.

Fig 136

Fig 1

× 150

× 150

Fig 140.

l   *l*

× 150

Fig 1

× 150

Fig. 139

l

× 150

l

× 150

axis

axis

Fig 143

Fig. 144

× 150

Fig 145

× 20

l   *ax.*   *l*
sy.   *sy.*

Fig 152

Fig 153

Fig 154

Fig 15

a   b   c

—sy
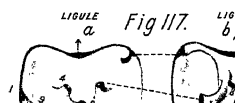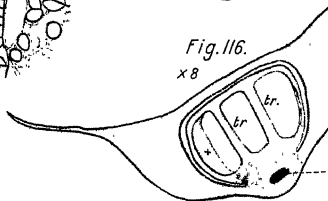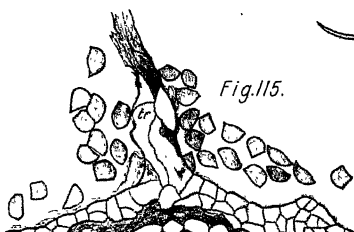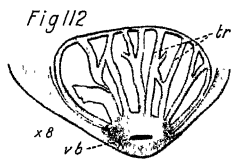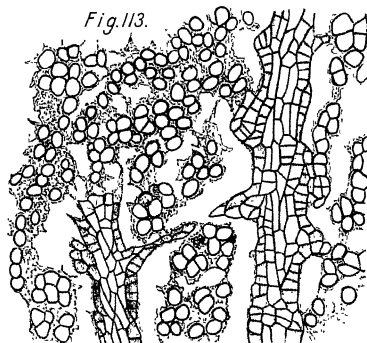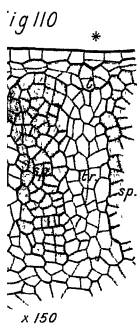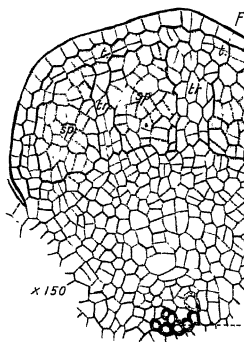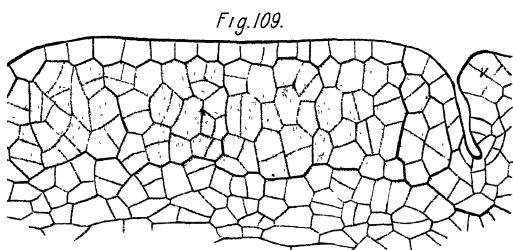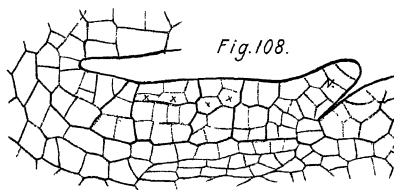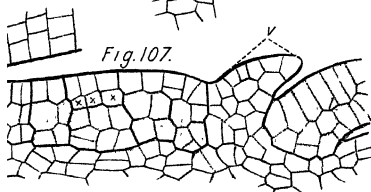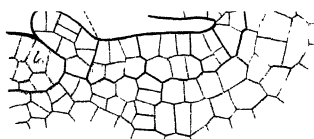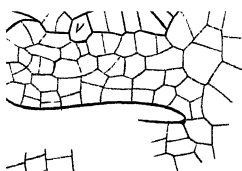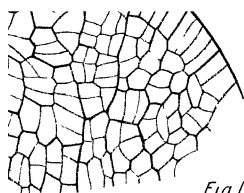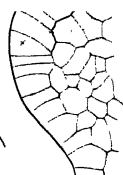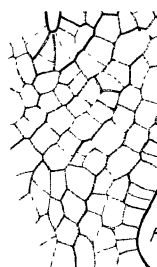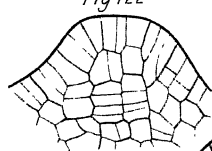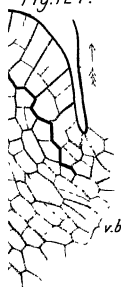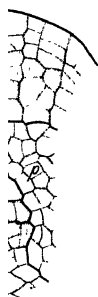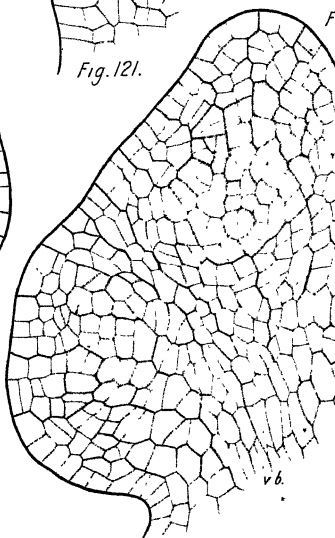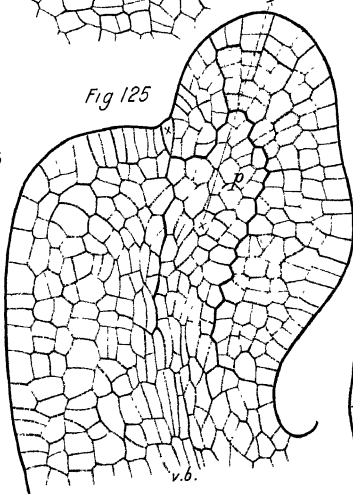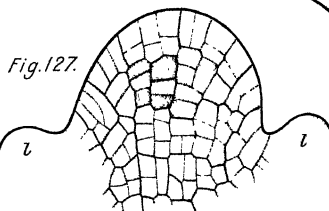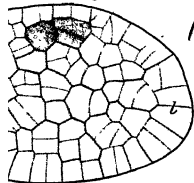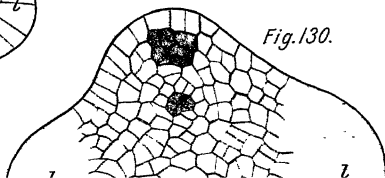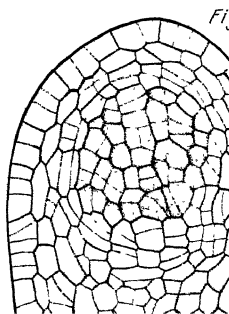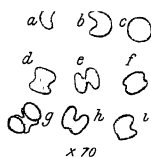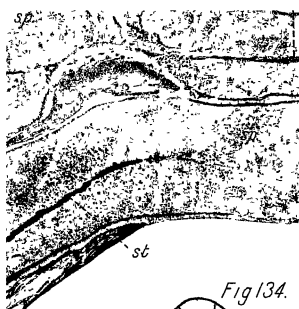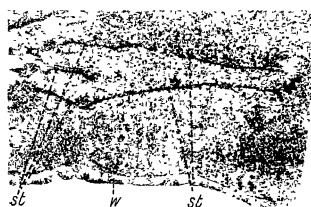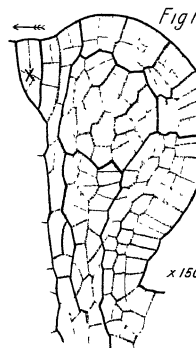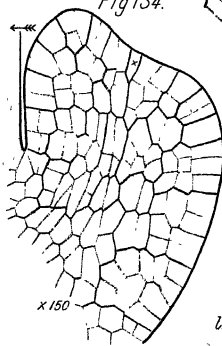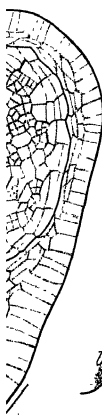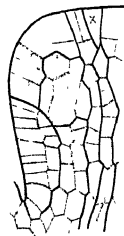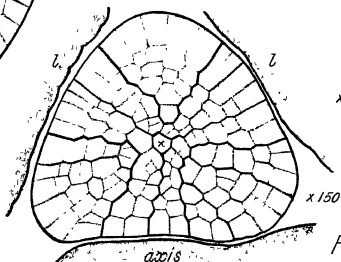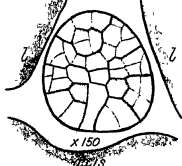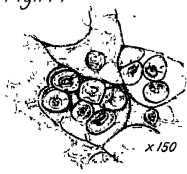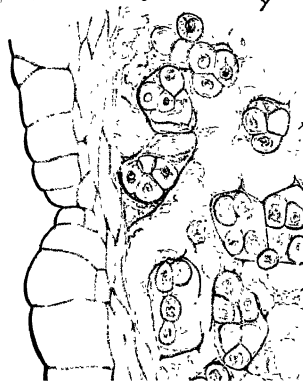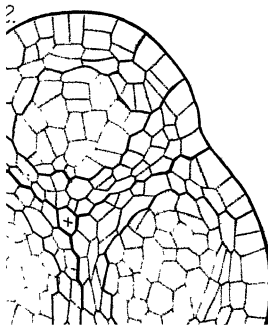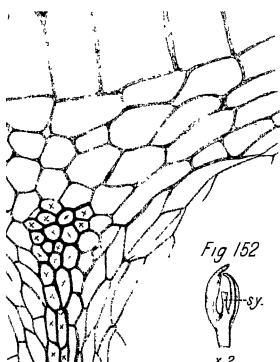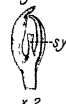
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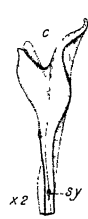
Fig 157   Fig 158   Fig 159.   Fig 160

Fig.162
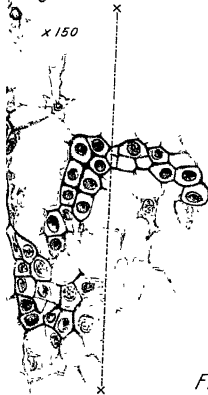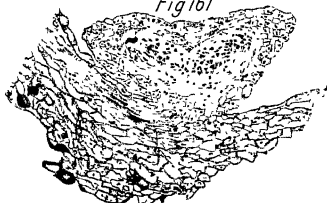
Fig 165.

Fig 161

Fig 163
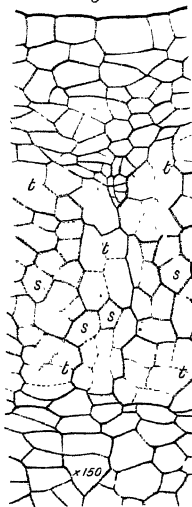
Fig.164.

Fig 167.
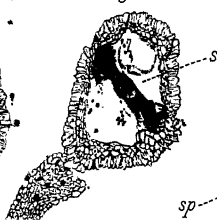
Fig.168

XIII. *Reptiles from the Elgin Sandstone.—Description of Two New Genera.*

By E. T. NEWTON, *F.R.S.*

[PLATES 53–56.]

CONTENTS.

I. INTRODUCTION.

SINCE the reading of my previous paper (68*), 'On some New Reptiles from the Elgin Sandstone,' several additional specimens have come to me for examination. They are all from the Elgin Sandstone, but, with possibly one exception, are from the quarry at Spynie, two miles north-east of Elgin. Two of these specimens are new forms, representing two new genera, and form the subject of the present communication. One of them, like all those in the earlier paper, is in the condition of hollow moulds, and a similar mode of investigation has been resorted to, namely, the preparation of gutta-percha casts from the cavities. In the second specimen the bones were present, but in a very friable condition; the skull, however, owing to the care with which it was uncovered by Mr. RICHARD HALL, of the British Museum, is now beautifully preserved, and most of the caudal vertebræ are still *in situ;* but the greater part of the rest of the skeleton had hopelessly crumbled away with the breaking open of the stone, and, for these parts, the casting process had again to be resorted to before the forms of the bones could be seen.

* These numbers refer to the List of Works, p. 603.

4.9.94

II. DESCRIPTION OF SPECIMENS.

1. ERPETOSUCHUS GRANTI, *gen. et sp. nov.* (Plate 53.)

General Remarks.

The first specimen to be noticed is contained in a small block of sandstone, the property of Mr. JAMES GRANT, of Lossiemouth, who has been good enough to place it in my hands for development and description.   The exact locality from which it came is uncertain, as it had been used for part of a breakwater before its value was discovered ; but there is no doubt as to its being from the Elgin Sandstone, and in all probability it came originally from the quarry at Lossiemouth, or that at Spynie. When the block first came to me it had been broken across in two directions, and one piece was wanting.   The two portions remaining formed an irregular cube, showing on one side some small cavities, which proved to be parts of the vertebral column that had been broken across.   Another surface, exposed by the breaking open of the stone, exhibited several holes, the meaning of which could only be conjectured, and an outline which seemed to indicate a transverse section of a skull.

These remains, like those from Cuttie's Hillock, are, as already mentioned, only represented by hollow cavities ; in the present instance, however, this is probably due to weathering since the stone was removed from the quarry.

In order to reproduce the forms of the bones, it was necessary, as in previous instances, to split open the cavities, and then, by chiselling away such parts as could be best spared, so to expose the hollow moulds that casts could be prepared from them.   After carefully probing to find in which direction the bones lay, for at this time the nature of the specimen was very uncertain, it was decided to split the smaller block from end to end, so as, if possible, to open longitudinally a cavity which seemed to be part of a skull.

This operation was successful beyond expectation, for it displayed not only the impression of the hinder half of the skull divided nearly vertically and longitudinally, but also a good portion of the vertebral column, surmounted by a row of small pitted scutes.   Moreover, it was now certain that the anterior part of the skull was in the larger block of stone, and its direction was shown by the position of the hinder part, so that, after partly sawing through this larger piece of stone, it also was split open vertically ; and thus was displayed not only the front of the skull, but also part of a ramus of the lower jaw.   Further development revealed the pectoral arch, with both the fore limbs as nearly as possible in their natural positions.   It was now evident that the skeleton must have been entire when embedded in the sand, and the lost portion of the block of stone doubtless contained the greater part of the body, with the hind limbs and tail, of this exceedingly pretty little reptile, the skull of which is only three inches in length.

Description.

Skull.—In the descriptions which follow, it will be understood that, for convenience sake, the casts are alluded to as if they were the original bones.

There can be no doubt that the skull, and, indeed, all the parts of this skeleton which have been preserved, have a very crocodile-like appearance, although in certain points it differs widely from any living crocodile and can only be compared with the Triassic *Parasuchia*.

Seen from above (Plate 53, fig. 2), the skull is very regularly pear-shaped, being broad and rounded in the temporal region, somewhat narrowed posteriorly, more rapidly narrowed in front of the orbits, but enlarging again near the front, before terminating in a rather pointed anterior extremity.

In this view, eight distinct openings are seen, namely, a pair of proportionately large supra-temporal fossæ, the still larger and almost circular orbits, a pair of elongated pre-lachrymal fossæ, and, quite anteriorly, a pair of small nasal apertures. The infra-temporal fossa is also just visible on the side in this view, making in all ten openings. Very little can be seen of the sutures, but there is no doubt as to the general position of most of the bones, although their exact extent is uncertain.

The small anterior apertures, which are doubtless the external nares, are bounded in front, and partly separated from each other, by a small bone (or pair of bones) which is evidently the united premaxillæ. This bone has a somewhat greater extent on the oral aspect (fig. 3), and gives evidence of having supported three or four teeth on each side. How much of the dentary margin was formed by the premaxillæ is not certain, but the notch, seen most distinctly on the left side, although to some extent due to imperfection, apparently marks the division between it and the maxilla; the latter being expanded at its front part and carrying larger teeth. The median, upper process of the premaxillæ is pointed and wedged into a cleft in the anterior end of the large flat bone, which forms the posterior boundary of the nasal apertures and nearly the whole of the preorbital upper surface; no definite median suture can be seen, but this area is almost certainly formed by the two nasal bones. Towards the front a groove is seen passing downwards on each side just behind the nasal aperture, which is clearly part of the naso-maxillary suture, and the sharp thread-like line, passing backwards from this suture nearly to the orbit, divides the upper from the side walls of this part of the skull, and, apparently, also marks the division between the nasal and maxillary bones. While the nasal bones are nearly smooth, the frontal, or interorbital region is marked by irregular pittings, which anteriorly become ridges and grooves that terminate rather abruptly, and seemingly mark the junction of the frontal and nasal bones, but no suture is visible. Posteriorly the rugosities of the frontals also end abruptly; the inter-temporal, or parietal region being almost smooth, and a little below the level of the frontal bones; this region has a median longitudinal crest which posteriorly joins the transverse ridge that marks the hinder boundary of

the upper surface and the upper margin of the occiput. The transverse ridge extends outwards on each side to the squamosal region, and seems to be formed chiefly by the parietal bones, which form a comparatively broad band of bone on the upper surface behind the supra-temporal fossæ, and extend for a short distance on the occiput.

There is no trace of a pineal fossa.

A side view of the skull (fig. 1) shows the same five vacuities that were seen from above ; but the infra-temporal and pre-lachrymal fossæ are here better shown, while the supra-temporal fossa is only just visible. As already mentioned the premaxilla is small, and seemingly is restricted to the anterior half of the narial opening, the thickened dentary margin which supports the larger teeth, apparently belonging to the maxilla. The whole of the side wall of the skull, between the anterior nares and the orbit, forms a deep and clearly-defined depression, marked off from the upper and lower surfaces by sharp ridges, which meet and form a rounded front a little behind the nasal opening. The upper part of this depression is occupied by a thin, wrinkled plate of bone, which partly separates it from the internal cavity ; and the appearance of this, together with the sharply-defined margins, makes it probable that the space was occupied by some soft tissue, possibly a gland. A large vacuity at the lower part opens into the internal cavity of the skull. The front part of this fossa is doubtless formed by the maxilla, as well as much of the flattened surface, seen on the under parts ; but how far it extends backwards is not seen, and probably the hinder portion of this depressed area and the front margin of the orbit are due to prefrontal and lachrymal elements, the sutures not being visible.

The upper and side aspects of this skull, behind and including the orbits, have so close a resemblance to the same parts in *Teleosaurus*, that it is in the highest degree probable that the bony elements, entering into its construction, occupy the same relative positions, but there are very few indications of sutures to mark their boundaries. Judging from the position of the post-palatine vacuity (fig. 3), the maxilla seems to extend backwards to about the middle of the orbit (fig. 1), from which it is probably excluded by the jugal. The sub-orbital bar divides posteriorly into two branches which form the upper and lower boundaries of the infra-temporal fossa. The lower branch becomes much attenuated, and its pointed end seems to be received into a socket of the bone which forms the lower and outer part of the pedicle supporting the lower jaw ; the pointed bar is evidently the jugal bone, and the socket receiving it is as certainly formed by the quadrato-jugal ; the latter bone probably forms the outer border of the pedicle, with the quadrate altogether on its inner aspect. The upper branch of the sub-orbital bar, passing backwards, unites first with the post-orbital bar, and then with the bones of the squamosal region to form the supra-temporal bar, to which is likewise attached the upper end of the pedicle for the lower jaw. The hinder end of the squamosal is pointed and free, and is not in close relation with the exoccipital process, as it generally is in crocodiles.

The quadrate is broken, but something of its structure may still be deciphered.

There is a single bead-like occipital condyle, with a pit in its middle, and above this the foramen magnum is indistinctly seen. On each side a large process passes off from the exoccipital region, and that of the right side is seen to extend upwards and outwards, so as to overhang somewhat, and come into close relation with, the quadrate articulation. This right exoccipital is obscured by two projecting pieces of bone which, as there is nothing to correspond with them on the left side, are believed to be accidental, and probably are portions of the atlas vertebra pressed out of place against the exoccipital. Above the foramen magnum the bone is much broken, and there is a considerable space without bone below the upper or parietal margin of the occiput. It seems probable that the supraoccipital is wanting, and that the bone in place above the foramen is to be attributed to the exoccipitals, which most likely meet above the foramen magnum.

When clearing away the matrix, the impression of a bone was seen passing upwards from the side of the cranial cavity, somewhat in front of the exoccipital to join the inner and upper part of the quadrate and also the squamosal. The wide upper part of this bone, in a back view of the skull, is above and in front of the exoccipital process. On comparing these parts with the same region in a crocodile, it is clear that this widened part of the bone must have formed the front wall of the auditory passage through which the columella passed. In the fossil, however, in its present condition, the passage is not completed behind, as it is in the crocodile, by the meeting of the squamosal with the quadrate and exoccipital processes.

The Palate of this skull (fig. 3) differs widely from that of any recent crocodile, but approaches the condition found in the Triassic *Parasuchia*. The greatest peculiarity is found in the forward position of the posterior nares, and in the deep trough which occupies nearly the entire length of the middle region. The roof of the mouth near the premaxillæ and the thickened parts of the maxillæ, that is to say, the part surrounded by the teeth, is slightly arched; but nearly opposite the hindermost tooth, the palate is suddenly deepened and a sharply defined trough is formed, which extends to the hinder end of the palate, and may indeed be said to reach to the basioccipital condyle. This trough is a little wider in the middle than it is at either end; anteriorly, the sharp overhanging edges appear to be formed by the maxillæ, these bones probably extending back as far as the post-palatine vacuities (*pt. pl.*), which lie close to the sides of the hinder part of the trough. It is probable that both palatines and pterygoids help to form the inner boundaries of the post-palatine vacuities.

On each side of the trough and extending backwards from the hindermost tooth, there is an edentulous, flattened edge almost certainly formed by the maxilla, which looks a little inwards and widens at its hinder end, where it embraces the front of the post-palatine vacuity. On the outer sides of these edges the under surface of the skull forms a wide obliquely flattened area which slopes outwards and upwards

to constitute the sharp lower margins of the pre-lachrymal depressions, seen on the sides of the skull.

This oblique area is narrow just behind the teeth (5 millims.) where it is probably formed entirely by the maxilla, but widens as it passes backwards, its greatest width being opposite the post-palatine vacuity, and directly below the orbit, at which part it is probably formed chiefly by the jugal, but no suture between this bone and the maxilla is visible. The posterior nares are placed quite at the front part of the median trough and below the front half of the pre-lachrymal vacuity; each is about 13 millims. long and 3·5 millims. wide, and is partly hidden in figure 3 by the overhanging sides of the trough. There is no reason for doubting that the outer and front walls of the posterior nares are formed by the maxillæ, and it is probable, though not certain, that the premaxillæ are shut out by the meeting of the maxillæ in the middle line.

The median part of the trough is occupied by a narrow spindle-shaped area, with a thread-like border on each side, which extends backwards nearly to the hinder part of the pterygoids, where it ends in a point, and forwards, between the posterior nares, for at least two-thirds of their length. If this area is formed by a distinct bone it can only be the vomer, and it is uncertain whether it is separated from the posterior nares by forward processes of the palatines or pterygoids. It is by no means clear what share the two bones last-named take in the formation of this trough.

In both crocodiles and lizards the post-palatine vacuity is bounded behind and outwardly by the transverse bone, which unites by a ⊤-shaped piece with the maxilla and jugal, while the stem is applied to the descending process of the pterygoid. A precisely similar bone, occupying a similar position, is seen in the present specimen, and is without doubt a transverse bone. Passing backwards from this the pterygoids are seen as strong ridges, extending to, and abutting upon the basisphenoid, where they are united in the middle line, leaving no indication of any inter-pterygoid foramen, such as may be seen in *Sphenodon* and *Phytosaurus* (*Belodon*). The pterygoids, passing forwards, probably form part of the post-palatine vacuity, and they seem to extend along the sides of the vomer, perhaps to the posterior nares, but it is most likely that the palatines form the posterior, and to some extent the inner, boundaries of these apertures. The palatines seem to form the side walls of the trough, and are, therefore, largely hidden in this view from below; they most likely form part of the inner wall of the post-palatine vacuity, and extend forwards to the posterior nares; but the absence of sutures leaves these points uncertain.

To return to the pterygoids, close to their junction with the basisphenoid, each gives off a large process, backwards, outwards, and upwards to the quadrate. This process, at its origin, is compressed from side to side, but is comparatively deep; this condition is speedily reversed and the process becomes a wide and depressed bone,

its broad outer end being applied to the under surface of the quadrate. In all probability much of the outer part of this bone is a process from the quadrate, but no suture is visible to divide it from the pterygoid. Each pterygoid, therefore, is seen to have the characteristic triradiate form, one short limb passing forwards and outwards to the transverse bone, a second large and broad process extending backwards to the quadrate, while a third process seemingly extends forwards along the trough of the palate.

The palatal surface of the vomer (?) is marked by irregular pits, similar to those which usually give attachment to cartilage, and it seems possible, therefore, that in the living animal there was a median cartilaginous septum; and this leads to the surmise, that possibly the sharp overhanging sides of the trough gave attachment to soft tissues, which may have separated this trough more or less completely from the general cavity of the mouth. If this were the case, the posterior nares, in the living animal, must have been thrown far back, as in recent crocodilia, but with the two nasal passages formed by soft tissues, and not by bone.

The *base of the cranium* between the occipital condyle and the pterygoid bones, gives no indication of its division into basioccipital and basisphenoid; it is concave both from before backwards and from side to side. A little in front of the condyle, and evidently formed by the basioccipital, there is a pair of rounded processes projecting almost directly downwards, and from each of these a ridge passes forwards and becomes a long laterally compressed and downwardly directed process at the front part of the basisphenoid. Just in front of these processes, the pterygoids are attached to the base of the cranium.

Lower Jaw.—Impressions of both the rami of the lower jaw are preserved, but to display either of them from end to end would endanger other parts of the head. Nearly the whole of the left ramus is seen, and this shows the form from the front to behind the lateral vacuity; while the hinder part of the right ramus is uncovered as far forward as the middle of the lateral vacuity. The form of the entire ramus, therefore, is known and is represented in the figure (Plate 53, fig. 1), the front part being drawn from the left side (fig. 4), and reversed. The entire ramus measures 87 millims. in length, and 10 millims. in height at its deepest part, that is near the hinder part of the lateral vacuity. The lower jaw, corresponding with the form of the skull, is narrow in front, but the rami diverge considerably as they pass backwards. The symphysis is comparatively long, and seems to extend for perhaps 23 millims. from the front. The teeth are restricted to the anterior third of the ramus, and eleven may be counted on the left side. The external lateral vacuity commences at 39 millims. from the front, and is 22 millims. long, while the articulation for the quadrate is 14 millims. behind this vacuity, and the hinder extremity extends for another 14 millims. backwards. Near the hindermost teeth the outer part of the dentary border begins to form a lateral ridge, which becomes better defined as it passes backwards, and overhanging the hinder part of the lateral vacuity, divides this region of

the outer surface into two oblique areas, an upper and a lower, which terminate a little in front of the articulation.

Although the sutures are not all very distinct there are sufficient to show that the usual elements are present. The articulare extends on the inner side quite down to the lower margin, and appears on the outer surface (fig. 1), where an impressed line, extending almost to the lateral vacuity, marks its union with the angulare; the latter bone occupying nearly all the triangular area seen, in a side view, below the articulation. The surangular extends from below the articulation to the lateral vacuity, and over this possibly to near its anterior extremity, being itself overlaid by the backward process of the dentary. The lower process of the dentary passes below the lateral vacuity, but at what point it meets the angulare is uncertain. There are indications of a splenial element extending forwards to within 15 millims. of the front of the jaw, but its hinder extremity is not shown.

Dentition.—The teeth are irregular and vary considerably in size; but they are all slender, conical, and recurved; their mode of implantation is not clearly shown, but the evidence, so far as it goes, is in favour of their being in distinct alveoli. The largest teeth are 5 or 6 millims. long and 2 millims. thick at their bases; they are found in the upper jaw in the thickened part of the bone which I have above referred to as the maxilla. Two of these large teeth are to be seen on each side; they are directed downwards, backwards, and but little if at all outwards. From the positions of these teeth, and from the appearance of the alveolar border, there seem to be spaces for four of these large teeth on each side, but possibly they were never all in place at one time. Two smaller teeth (about 2 millims. long) are preserved on the right side nearly at the anterior extremity, and evidently in the premaxilla; they are, perhaps, turned a little more outwards than the large teeth. On the left side the alveolar border of the premaxilla is best seen and this seems to show spaces for three or four teeth, all smaller than those of the maxilla. I have been unable to find traces of teeth or alveoli behind the large teeth of the maxilla, although the more extended dental series of the lower jaw led one to expect that corresponding teeth would be found in the upper jaw.

Eleven irregularly spaced teeth may be counted in the left mandibular ramus within 25 millims. of the anterior extremity. They differ much in size, but none are quite so long as the largest ones of the upper jaw, and some are very small.

Pectoral Arch.—The impressions of the greater part of both scapulæ, the two coracoids, and the upper surface of the interclavicle have been exposed; they occupy very nearly their natural relations to each other, but the scapulæ have possibly been pressed down into a more horizontal position than they naturally held.

The *scapula* (figs. 9, 9A) is an elongated slender bone, rod-like in the middle, ... and expanded above, and curved much forwards at its lower end, where it .... an arched plate, the concavity of which looks downwards and .... of this plate forms a strong ridge, and apparently corre-

sponds with the process found in a similar position in the scapula of the crocodile. The lower edge of this plate is thickened and rough, and is, in part, attached to the coracoid, but a space is left between the two bones which was evidently occupied by cartilage ; posteriorly this space widens, and both the bones become thicker, thus forming the glenoid cavity. A little above the glenoid articulation (7 millims.), on the inner aspect of the scapula (fig. 9A), there is a distinct prominence which appears to be for the ligamentous attachment of a clavicle, and there is some evidence that such a bone existed.

The length of the scapula from the glenoid cavity to the upper extremity is 32 millims. From the same point to the anterior extremity 11 millims. Diameter of shaft 3 millims. Width at upper extremity 6·5 millims.

Each Coracoid (fig. 9) is a small quadrate bone about 10 millims. from front to back, and 7 millims. wide; it is deeply concave above and convex below, with a straight and thickened inner edge. The impressions of both coracoids are seen lying just above the inter-clavicle (fig. 14) and very nearly meeting in the middle line ; but they are a little out of place, and the thickened edge of the right one looks more backwards than inwards, this, however, is partly due to displacement. It is tolerably evident that the inner edges of the coracoids were naturally placed at an angle to each other and were attached either to the oblique front edges of the inter-clavicle, or to a plate of cartilage in relation with the inter-clavicle. The coracoid (fig. 9) seems to be attached to the scapula at two points, with an opening between ; but whether this is accidental, or represents the coracoid foramen, is uncertain.

The inter-clavicle (fig. 14) is attenuated at both extremities, it is about 35 millims. long, and anteriorly has the form of an arrow-head, the widest part of which is 9 millims. The front is curved a little upwards and its end is bead-like ; for a short distance behind this there is a median raised line, and then follows a shallow depression extending nearly to the hinder end. The probable relation of this bone to the coracoids has been alluded to above.

On the left side, lying in close relation to the scapula, coracoid, and inter-clavicle, there is a curved bone which is very suggestive of a clavicle, but the evidence is insufficient for any definite interpretation.

Humerus.—The impressions of the greater part of both humeri are preserved (Plate 53, figs. 10, 10A, 12), and with the exception of the distal articular condyles, the form of the bone is well shown. The greatest length is 38 millims.; the width of the proximal end, including the pectoral crest, is 9 millims., and the distal extremity measures as nearly as possible the same ; while the middle of the shaft has a diameter of scarcely 3 millims. The bone is somewhat twisted so that the expansions of the two extremities are not in the same plane. The proximal articulation is compressed, and seems to be continued by a ridge into the large pectoral crest, which extends down the shaft to about 16 millims. from the proximal end of the bone. The upper surface (fig. 12) in this region is convex from side to side, and the downward projection

of the pectoral crest makes the lower surface (fig. 10) deeply concave. The middle of the shaft is almost cylindrical. The upper aspect (fig. 12) of the expanded distal extremity is marked by a triangular depression, which is continued between the articular condyles, the latter appearing to be very definitely divided ; but this part is somewhat obscured by the upper end of the tibia. The under surface (fig. 10) of the distal expansion is convex, but this gives place to the groove between the condyles ; it is in this part that the humeri are most defective, both condyles being absent from the bone of the left side, and only one is shown on the right.

Radius and Ulna.—The right and left bones are both represented (figs. 11, 13), but on neither side are they quite perfect, the proximal end in all cases being defective. Sufficient, however, is preserved on the right side to show that both radius and ulna were about 30 millims. long and nearly straight, the latter being larger than the former. Proximally the ulna is flattened from before backwards, and the radius is rounded, while distally both bones seem to be laterally compressed.

Fore-feet.—Portions of both the fore-feet are present (figs. 11, 13), that of the left side being the more perfect. It is the plantar surface of the left foot (fig. 11) which is preserved, and five metacarpals may be seen very nearly in their natural positions, the middle one seeming to be the longest. Only a few of the phalanges could be traced, although it is probable they were all present, but the coarse matrix prevented their being successfully uncovered. It is the upper surface of the right foot (fig. 13) which is shown, and here also the five metacarpals are present, but they are somewhat displaced.

Carpal bones are preserved ; but, in spite of numerous casts and very close examination of the impressions left in the stone, I am unable to satisfy myself as to their number. There seem to be four cavities in the stone, one or two of which may be the ends of the metacarpals. The cast taken from these cavities may be interpreted as two, or perhaps three, ossicles in a proximal row, and one between these and the middle metacarpals ; but the number is uncertain, and the settlement of this point of structure must await the discovery of a more perfect specimen.

Vertebræ.—Immediately behind the head are the impressions of a series of vertebræ, which have been split open vertically and longitudinally. The twelve anterior vertebræ are present in a more or less perfect condition, and (excepting the first) the casts taken from their impressions give a very satisfactory knowledge of their structure ; parts obscured on one side are shown on the other ; for example, the centra and neural arches are well seen in several instances on the left side (fig. 5), but the neural spines are hidden by the scutes which have been pressed down upon them. On the right side, however (fig. 6), the neural spines and articulations of all the vertebræ, from the second to the sixth inclusive, are clearly seen. There are only fragments of the tenth, eleventh, and twelfth vertebræ.

When speaking of the occiput, p. 577, mention was made of some fragments, probably parts of a vertebra, pressed against the exoccipital ; these are most likely parts

of the first or atlas vertebra, but they are too indistinct to give any idea of their form. All the succeeding vertebræ, so far as can be seen, are bi-concave. The second or axis vertebra (fig. 6) has the neural spine much elongated from before backwards (10 millims.), and it overlaps the greater part of the succeeding vertebra ; the neural arch also extends a little in front of its own centrum, and was doubtless in close relation with an odontoid bone, not now in place. The centrum is slightly concave in front, for articulation with the odontoideum, and rather more concave behind. The sides of the centrum are pinched in so as to form a sharp and deep median keel, and this character is repeated in all the centra which are preserved. The third vertebra has the neural spine narrow from before backwards and almost pointed above, while those of the succeeding vertebræ gradually increase a little in antero-posterior extent. The neural arch presents, in a side view, a wide, flattened, trapezoidal area, the upper angles of which form the anterior and posterior zygapophyses, and these articulations are particularly well shown on the right side.

The union of the neural arches with the centra is best seen on the left side, where the sutures may still be traced. Similar neural arches are found as far back as the sixth vertebra, but with a slight increase in size. The sixth vertebra shows, just above the neuro-central suture and near the middle of the centrum, a tuberosity, which corresponds in position with the upper articular process for the cervical rib found on the sixth vertebra of a young alligator used for comparison, but it is proportionately smaller and does not form a distinct process.

The seventh and eighth vertebræ are broken and partly hidden, but the ninth (figs. 5 and 7) shows the upper articulation for the rib, well up on the side of the neural arch, while a lower articular surface is seen just below the neuro-central suture and near the front of the centrum ; both these articular surfaces agree with those found in the corresponding ninth vertebra of the alligator.

The vertebræ behind the ninth are indistinct, but portions of two dorsal ribs are present, and they both have double heads. It is clear, therefore, that each of the anterior dorsal ribs had a double articulation with its vertebra, and that the positions of the articular surfaces of the ninth vertebra correspond with those of the ninth vertebra of the alligator. It is also evident that, passing forwards, the upper articulation descends to the neuro-central suture on the sixth vertebra ; while this suture and the upper articulation encroach more and more upon the centrum from the fifth to the second vertebra. Near the front part of the centrum of each of the anterior three or four vertebræ a slight rugosity is seen, which may be an articular surface for the lower process of the cervical rib ; but as the sixth vertebra does not show any such lower articulation, it is uncertain whether or not the cervical ribs had double articulations. I have been unable to trace any of the cervical ribs.

Scutes.—A continuous series of closely opposed scutes is seen extending, in the region of the neural spines, from the occiput as far backwards as the specimen is preserved. In no part can more than two scutes be certainly seen side by side, and

it seems probable, therefore, that there were only two longitudinal rows in the cervical and early dorsal regions; but as the scutes, which are seen, are all on the left side of the neural spines, and it is possible that others may be hidden in the matrix, one cannot speak with certainty. All the scutes are quadrate and longer than they are wide, the proportion being about three to two, one of the hinder ones measuring about 7·5 millims. by 5 millims.; each has a longitudinal ridge, which is rather nearer the outer than the inner margin; and the exposed surface is ornamented by distinct pits, which are rounded near the middle of each scute, but more or less elongated towards the edges. These pittings have a tendency to radiate from the middle of the scute.

On the outer side of the hindermost pair of scutes (that is the ninth pair counting from the front, and overlying the eleventh or twelfth vertebra) there is a fragment which looks like a piece of another scute, and this may possibly indicate an increase in the number of rows of scutes in the dorsal region.

Affinities.

The general form of the upper part of the skull of this specimen agrees so closely with the crocodilia generally and with *Teleosaurus* in particular, that there can be little question as to these parts being constructed upon the same plan. The form and position of the four cavities, so characteristic of the skulls of ordinary crocodiles, namely, the two supratemporal fossæ and the orbits, are well exhibited in this fossil, and there is a similar narrowness of the frontal and parietal regions. The small external nasal apertures are placed near the front extremity, but are divided by bone in our fossil, although usually undivided in recent crocodilia. In all these particulars, except the last, *Teleosaurus* approaches nearer to our fossil than do the recent forms. The infra-temporal fossa seen on the side of this skull is found also in the recent crocodiles, but in them it is not so distinctly divided from the orbit in front and from the auditory channel behind. In both these particulars, again, *Teleosaurus* more nearly resembles this fossil, as it does also in the position of the quadrate pedicle. The large pre-lachrymal vacuity finds no counterpart in the recent crocodilia; it is present in *Teleosaurus*, although much smaller; but the latter genus has no similar depression of the side walls in the vicinity of the vacuity.

If only the upper part of this little skull were known, the palate being hidden, there would have been but little hesitation in regarding it as a close ally of *Teleosaurus*; but the fortunate preservation of so many important features of the palate prevents any such reference. The forward position of the posterior nares gives the palate a very lizard-like character, and is quite unlike any form of *Eusuchia* or *Mesosuchia*, but resembles the condition found in that of the Triassic *Parasuchia*, as described by Professor HUXLEY in his memoir on the Elgin *Stagonolepis* (17, 18). Unfortunately, the skull of the latter genus is very imperfectly known, but still

sufficient is preserved (18, Plate 9) to show that there are important resemblances, as well as differences, between it and the present specimen. Professor Huxley's figures show a similar divergence from the living crocodilia in the forward position of the posterior nares, and absence of any inward growth of the palatines and pterygoids to form complete bony channels, such as are found in more recent forms, and which throw the posterior nares so far back. The possibility that, in the present specimen the nasal passages were carried further back by soft tissues, has been noticed on p. 579. Another important resemblance between this skull and that of *Stagonolepis* is the presence of the large pre-lachrymal vacuity. Professor Huxley pointed out the affinity with *Belodon* (*Phytosaurus*) which both these peculiarities indicate. *Stagonolepis* has two troughs along the palate, separated by a prominent median ridge, formed by the pterygoids and vomers, while in the present specimen there is but a single and much narrower median trough, the vomers and pterygoids not being prominent, but forming, apparently, the bottom of the trough. The relation of the orbit to the pre-lachrymal vacuity and to the infra-temporal fossa, as well as the width of the inter-orbital space, is very different in the two forms.

The teeth of *Stagonolepis* are short and stout, with inflated but somewhat compressed crowns, and differ, therefore, in a marked manner from the slender, tapering, recurved teeth of the present specimen.

If comparison be made with *Phytosaurus* (65, 66, 18), similar pre-lachrymal vacuities will be found, and the relation of these to the orbits and to the anterior nasal apertures will be seen to closely resemble the arrangement of the corresponding parts in our Elgin fossil. It is the enormous development of the premaxillary region in *Phytosaurus* which makes the two skulls so unlike in general appearance. The supra-temporal fossæ in the last-named genus, are not seen as distinctly circumscribed openings on the upper surface of the skull, and consequently the hinder part of this region has a different aspect in the two forms.

The palate of this Elgin skull bears a closer resemblance to that of *Phytosaurus* than to that of *Stagonolepis*, for *Phytosaurus* has a single median trough, and the vomers and pterygoids do not form a median ridge as they do in *Stagonolepis*. The pterygoids in *Phytosaurus* and in the present specimen have a similar relation to the basisphenoid; in both this bone has a backwardly-directed quadrate process, resembling more the lacertilian than the ordinary crocodilian type of structure. In the Elgin skull, however, the pterygoids have completely united in front of the basisphenoid, but in *Phytosaurus* there is an inter-pterygoid foramen, and this is important, as indicating a nearer approach to the lacertilian and rhynchocephalian condition of the pterygoids than is found in any ordinary crocodilian; indeed the resemblance between this part of the palate in *Phytosaurus* and in *Sphenodon* is remarkable.

The pectoral arch of this Elgin specimen differs from that of the ordinary crocodilian in its more elongated scapula and its shortened coracoid, but in these parti-

culars again it resembles *Stagonolepis* and *Phytosaurus,* and seemingly approaches the lizards, a shorter coracoid being generally characteristic of the latter group, and the elongated scapula is paralleled in the chamæleon. The fore limbs conform to the crocodilian pattern, but it is possible that the carpus may prove to be of a more generalized type. The vertebræ correspond in a striking manner with those of ordinary crocodiles, although it is not certain that there were doubly articulated cervical ribs, and the amphicœlous centra again link them on to the Parasuchia. The ornamented dorsal scutes also point to a similar relationship.

The above comparison shows most unmistakably that the specimen here described is more nearly related to the Parasuchia than to any group of living reptiles, but in the form of the upper part of the skull it approaches the mesosuchian genus *Teleosaurus.* And further, those points of its structure in which it differs from the Eusuchia and Mesosuchia, namely, the forward position of the posterior nares, the presence of a pre-lachrymal vacuity, the bi-concave vertebræ, the elongated scapula, and the short coracoid,—are just those points in which it resembles *Stagonolepis* and *Phytosaurus.* There seems no doubt, therefore, that our specimen is most nearly related to these two forms, and that its proper systematic position is with them in the Parasuchia, whether this group be retained as a division of the Crocodilia, or raised to a distinct order, as suggested by Mr. R. LYDEKKER (24, p. 235). It will be obvious, however, that the differences in the skulls prevent a reference of the present specimen to either of the above-named genera. It is separated from *Stagonolepis* by the structure of the palate, by the different arrangement of the openings on the upper surface, and by the form of the teeth. On the other hand, it is distinguished from *Phytosaurus* by its short premaxillary region, by the very different form of the temporal region and fossæ, by the proportionately large and differently placed orbit; also by the absence of an inter-pterygoid foramen, by the narrowly troughed palate, and by the restricted area occupied by its teeth.

I suggest for this reptile the name of *Erpetosuchus Granti.*

2. ORNITHOSUCHUS WOODWARDI, *gen. et sp. nov.* (Plates 54, 55, 56.)

General Remarks.

The second specimen to be described is one that was discovered a few years ago by the Rev. Dr. GORDON, in the reptiliferous sandstone at Spynie, near Elgin—a locality rendered classical by the discovery, in the year 1851, of the celebrated *Telerpeton Elginense.* The present specimen was sent by Dr. GORDON to the British Museum, and at that time showed the greater part of a vertebral column, with parts of the hind limbs; also a series of small scutes, and a lower jaw broken through horizontally so as to show several teeth in cross-section. The bones themselves, although present, were in an exceedingly friable condition, and, where broken through

by the splitting open of the stone, had largely crumbled away. The skull, which now forms a conspicuous and important part of the specimen, was at that time wholly concealed, and was discovered in one of the slabs under the lower jaw by Mr. RICHARD HALL, of the British Museum, who, with his accustomed skill, disengaged the greater part of the cranium from the matrix, and has thus brought to light one of the most perfect skulls yet found in the Elgin Sandstone. Few, even among those experienced in developing fossils, can appreciate the difficulties overcome by Mr. HALL in the delicate task of clearing away the hard sandstone from the decayed and friable bone without destroying the specimen itself.

This reptile was to have been described by Mr. A. SMITH WOODWARD, of the British Museum, who, when exhibiting it at a Soirée of the Royal Society, remarked on its possible affinities with the Triassic *Aëtosaurus* of Dr. FRAAS (56); but a plethora of work in other directions prevented his accomplishing this task, and hearing that I was engaged upon some fresh material from the same locality, he most generously gave me the opportunity of describing this new and interesting specimen. It is with much pleasure that I acknowledge my indebtedness to Mr. SMITH WOODWARD for his disinterested courtesy.

The specimen is now contained in two slabs of sandstone, each broken across, and several smaller pieces, all portions of one block which, on being split open, revealed the parts above mentioned. When the animal was buried in the sand the head was turned over on the dorsal region, with the nose turned backwards (Plate 54). The parts of the skeleton preserved being so nearly in their natural relations to each other, it is almost certain that the cervical vertebræ and fore limbs were also in place, but no trace of them is seen, and as the stone has been broken across just at the front of the thorax, there is but little doubt that these parts were contained in a piece of stone which has not been preserved.

The palate was still covered by matrix when the specimen came into my hands, but the desirability of knowing the structure of this region led me to undertake the hazardous task of uncovering it, and this has now been safely accomplished; a small part, however, still remains covered by the symphysis of the lower jaw, but to expose this would mean the destruction of the symphysis with several of the teeth. Some other parts of the skeleton have likewise been further cleared of matrix. Most of the pre-caudal vertebræ and limb bones were so broken by the original splitting open of the stone that only fragments of the bone remained, and it was necessary to clear out these fragments and take casts from the cavities in order to ascertain the original forms of the bones. It is these casts, therefore, which, for the most part, have supplied the material for the following descriptions, but the account of the skull is taken from the specimen itself, the bone being well preserved.

Description.

Skull.—The general aspect of the skull, when seen from above (Plate 55, fig. 2), is very bird-like, being broad at the back and pointed and beak-like in front. In a side view (fig. 1), however, the premaxillary region is seen to have a considerable vertical extent, and the nasal apertures are large and close to the front; moreover, the parietal region (fig. 2) is narrow, and there are distinct and completely enclosed supra-temporal fossæ. The greatest length of the skull, from the front to the quadrate, is 115 millims., the width across the quadrates 54 millims. The bone, although broken at the edges, is sufficiently well preserved to show the natural form. The sutures are well shown in almost every instance, and consequently the elucidation of the structure is comparatively easy, although the arrangement of some of the parts is peculiar.

The upper surface (fig. 2) has the bones in distinct pairs from front to back, and the various bones are at once recognizable. The small backward processes of the premaxillæ are wedged in between the front points of the nasal bones, and the latter join the frontals by a transverse suture well in front of the orbits. The parietals form an obtuse angle anteriorly, which is received between the hinder ends of the frontals. A median suture extends throughout the length of the upper surface of the skull. There is no parietal foramen. On each side, extending outwards from the hinder part of each parietal, is a band of bone, the upper edge of which forms the boundary between the upper and hinder surfaces of the skull; it also forms the posterior walls of the supra-temporal fossæ, and joins the squamosal on each side at the hinder and outer angle. This band is evidently formed by the parietals, no suture being visible, and it overlaps on each side a post-temporal fossa. The outer boundary of the supra-temporal fossa is doubtless formed by the squamosal, which joins the parietal band posteriorly, and extends downwards on the outer side of the skull (fig. 1), but no suture can be traced between this bone and that, evidently the postfrontal, which forms the hinder angle of the orbit, and is internally united to both the frontal and the parietal. The anterior angle of the orbit is also formed by a distinct bone, the prefrontal. At first sight this bone seems to extend forwards on the outer side of the large nasal bone (fig. 2), but there is a suture near the anterior extremity of the frontal bone cutting off this anterior part, which in a side view (fig. 1) is seen to be a forward extension of the lachrymal bone above the great pre-lachrymal fossa.

A side view of the skull is rendered striking by the large size of the pre-lachrymal fossa, by the peculiar form of the infra-temporal fossa, and by the formidable predaceous teeth. The orbit is large and narrow inferiorly, reminding one of the orbit of *Scaphognathus*. The large nasal apertures are near the front, and each is enclosed by its nasal bone and premaxilla; the junction of the latter with the maxilla is probably hidden by the large tooth projecting upwards from the lower jaw. The two largest teeth are evidently planted in the maxilla, which is seen to extend backwards

as far as the orbit, and to bear teeth nearly to its hinder extremity. The maxilla is excluded from the orbit by the jugal, which itself sends upwards two slender processes, one before, the other behind, the orbit, and thus forms its lower boundary. The anterior process extends upwards in front of a slender bone occupying the position of a lachrymal, which forms the front wall of the orbit, and is continuous with that seen above the pre-lachrymal fossa and on the outer side of the nasal bone (fig. 2). The posterior upward process of the jugal unites in a similar manner with the bone forming the hinder boundary of the orbit, which can only be interpreted as a postorbital; unless it be a long process of the postfrontal, by which its upper end is overlaid. The quadrato-jugal, which underlies the hinder process of the jugal, is one of the stoutest bones of the side of the skull; it forms the outer angle of the pedicle for the lower jaw, and, extending upwards on the outer side of the quadrate, joins the squamosal. The quadrato-jugal thus shuts off the quadrate from the side wall of the skull. The squamosal is T-shaped in this view, and seems to overlap the upper end of the quadrato-jugal; but there is a V-shaped suture a little above this, which may indicate a distinct bone. When viewed from behind, the quadrate is seen to form about half the width of the pedicle, being overlaid by the quadrato-jugal; the latter, however, is narrowed at its lower end, so that the articulation for the lower jaw is formed entirely by the quadrate, which, a little above its middle, is pierced by a large foramen. Extending upwards, the quadrate reaches the point where the parietal meets the squamosal, and expanding at its upper part seems to be in contact with the inner side of the downward process of the squamosal; but this part is somewhat hidden by the matrix. From the lower and front part of the quadrate a broad process passes forwards to join the pterygoid (fig. 3). The latter bone will be alluded to again, when describing the palate.

The Occiput (fig. 2) is much broken, but something of its structure may be seen. The upper part, as already noticed, is formed by the parietals, and immediately below this, in the middle line, is a four-sided plate occupying the position of a supraoccipital; but, from its lower part, on the left side, a process passes off to the point of the squamosal, and as there can be no question as to this corresponding with the exoccipital process of the crocodile, it is probable that much of the four-sided plate is formed by the exoccipitals meeting above the foramen magnum; and it may be that the supraoccipital is represented by the small triangular area at the upper part, although the line of separation looks more like a breakage than a suture. On the left side, between the exoccipital process and the parietal band, there is a distinct posttemporal fossa. On the right side part of the exoccipital bar is broken away, revealing a more deeply-seated bone, probably the opisthotic. There are traces of bone in the position which would be occupied by the basioccipital, but its form is by no means clearly defined, and its rounded end may not represent an articular condyle.

The Palate (fig. 3) being partly covered by the lower jaw, could not be wholly displayed, and about an inch of it still remains hidden by the symphysis. It is also

unfortunate that very little of the sutures can be made out. The quadrate bone sends forwards and inwards a large bar, which joins what is clearly a backward process of the pterygoid; anteriorly this process is united with a broad plate of bone that, with its fellow of the opposite side, extends backwards in the middle line to join the base of the skull, and spreads outwards in a broad wing; these parts are, without doubt, the pterygoid, which closely resembles that bone in the crocodile. From the anterior and outer part of this wing, and seemingly separated from it by a suture, a small bone passes to the point of union of the maxilla and jugal bones, and there spreads out into a T-shape; this must be the transverse bone; and the aperture seen in front of it is evidently the post-palatine vacuity. How far the pterygoid extends forwards, and how much of this broad plate is formed by the palatine, is uncertain. Close to the middle line, and between the post-palatine vacuities, is a pair of elongated apertures, the interpretation of which is not so satisfactory as could be wished. These apertures are regarded as the primitive posterior nares thrown far back, but they may be secondary nares, brought back to this position by the meeting of palatal laminæ of the palatine bones; or they may possibly be merely inter-pterygoid vacuities, such as are present in Dicynodonts. The front of the palate being hidden by the lower jaw, it is not certain but that the posterior nares are further forwards, as in lizards and in the form above named *Erpetosuchus Granti*.

The palate is divided throughout its length, so far as exposed, by a median suture, and the bones of the left side are pressed downwards a little, making the division very distinct. This suture runs along the bar of bone which divides the posterior nares. That the hinder median part of the broad plate which joins the base of the skull, and that passing back to the quadrate, as well as that united to the transverse bone, are all three parts of the pterygoid there can be little question; but we are left in doubt as to how far this bone extends forwards. If the line seen on the right side (on the left, fig. 3), passing between the front parts of the post-palatine vacuity and posterior narial opening, is really a suture, then in all probability it marks the anterior boundary of the palatine bone, as in *Phytosaurus* (66, Plates 38, 42). These median apertures seem most nearly to agree with the pair seen in the genus just named, which are referred to by Professor HUXLEY as the posterior nares (18, Plate 9, fig. 6), although in that form they are in advance of the post-palatine vacuities, as they are in all lizards. Among reptiles it is only in the crocodiles, where the secondary posterior nares are formed by the meeting the palatines, that they are thrown as far back as they are in the present fossil, and occupy a more posterior position than the post-palatine vacuities; but, excepting this backward position, there are no indications that these apertures, in the present specimen, are anything but primitive posterior nares; it is only their relative position which raises any doubt, and this to some extent finds a counterpart among birds. The longitudinal double bar of bone separating these two apertures may be formed by the palatines or by the pterygoids, but more probably by the vomers. In front of these apertures this median bar con-

tinues as a narrow double rod, separated from the broad plates on each side, which are almost certainly parts of the maxillæ ; and if it were quite certain that this separation were natural and not due to breakage, this pair of median elongated bones could scarcely be regarded otherwise than as vomers ; and it would also be pretty well demonstrated that the pair of apertures were the primitive posterior nares, an interpretation which is here adopted as most probably the correct one.

The Lower Jaw having been broken through longitudinally, and the upper margin being hidden by the maxillæ, its form cannot be properly seen, but the accompanying figure has been restored from the parts remaining and by careful measurements, so that it represents as nearly as possible the true form (fig. 1). The greatest length is about 110 millims. It extends some 7 or 8 millims. behind the quadrate articulation, but does not reach the front of the muzzle by perhaps 12 millims. At about 25 millims. in front of the hinder extremity there is a large lateral vacuity 33 millims. long, extending a little in advance of the orbit. The anterior half of the ramus is comparatively deep, but when seen from below (fig. 3) the hinder part of the symphysial region is found to be very narrow, the alveolar margin of this and all the hinder parts of the jaw being received within the teeth of the upper jaw. Anteriorly the rami are enlarged to carry the prominent teeth, which bite outside the upper jaw at the hinder part of the compressed premaxillary region, and below the posterior end of the nasal orifices. At the front each ramus carries one or more smaller teeth, which are received into the deep palate on the inner sides of the premaxillary teeth. The alveolar margin being hidden, the number of these teeth is not known. The hinder half of each ramus is stouter than the front part, and widens to form the articular surface for the quadrate. No sutures can be certainly traced.

Dentition.—The teeth, although partly broken and hidden by matrix, supply much information as to their form and arrangement (figs. 1, 3). They vary in size, but are otherwise similar in form, the largest being about 22 millims. in length. Each tooth is set in a distinct socket, and the larger ones have quite half their length sunk in the jaw ; but while the front ones seem to have been firmly fixed, the hinder ones appear to have been comparatively loose, some of the hinder maxillary teeth being more or less out of their sockets. Several of the teeth are still in place, as shown in the figures, and others have been broken across, showing spindle-shaped transverse sections. All the teeth, so far as can be seen, are recurved, pointed, and compressed, with acute anterior and posterior edges. Some of the larger teeth show the hinder edge of the crown to be serrated throughout its length, and the front edge for about half its length. The basal parts of the teeth are rounder than the crowns and are not serrated.

There appear to have been three moderate-sized teeth in each premaxilla, projecting about 3 or 4 millims. beyond the alveoli ; but there may have been others which are now hidden by the lower jaw, from which at this part a large tooth projects upwards outside the premaxilla. At the front of the maxilla there is a small tooth directed much

outwards, and following this on each side two large sabre-like teeth, passing down-wards and backwards, with only just room between the teeth of the two maxillæ to receive the narrow symphysis of the lower jaw. Much of the crowns of these teeth s broken away from the skull, but those of the left side are preserved in the opposite block of stone, and are shown restored in the figure. The roots of these two teeth are exposed by the breaking away of the outer lamina of the maxilla (fig. 1); the hinder one is the largest, its entire length being 22 millims., the crown projecting about 10 millims. from the alveolus (fig. 1A). The longest diameter at the base of the crown is 4 millims. Behind this largest tooth on the left side, six or eight alveoli may be seen, some containing broken teeth, and on the right side some of the teeth are present, but partly fallen from their sockets. The teeth decrease in size towards the back of the maxilla, and none of them was so large as the two preserved and figured.

At the front of the lower jaw there is evidence of two, perhaps three, forwardly directed teeth on each side, about equal in size to those of the premaxillæ, within which they bite, and they extend forwards to about the second premaxillary tooth. At the thickened part of each ramus there is the large tooth, which bites outside the premaxilla. One of these two teeth being further forwards than the other, it seems probable that there were alveoli for two of these large teeth in each ramus ; but possibly the four teeth were not in place at the same time. Behind the large teeth there are traces of others, but nothing definite, the alveolar border being hidden.

Vertebral Column.—The cervical vertebræ are wanting ; but thirteen pre-sacral, three sacral, and twenty-one caudal vertebræ are well preserved in an almost unbroken series, and supply all the important vertebral characters of these regions. The centra throughout are completely ossified, and the terminal faces of the centra are only slightly concave.

The *pre-sacral vertebræ* had been broken through in two or three directions, and so much of the bone had crumbled away that the small portions remaining were unintelligible, but, by clearing away the fragments and developing certain parts hidden by the matrix, it was possible to take casts which reproduce the greater part of both sides of the series, and the right side being the most perfect, is figured (Plate 56, fig. 1).

The centra are all as nearly as possible 11 millims. long, and 7 millims. high ; they are pinched in at the middle, but expanded at their articular faces, the edges of which are somewhat thickened ; inferiorly they all appear to be longitudinally keeled. The position of the neuro-central suture is shown in the first and fourth vertebræ from the sacrum, by the arches being raised from the centra, and in the thirteenth its place is marked by a depression. Throughout this series of pre-sacral vertebræ the rib articulations are both above the neuro-central suture. The neural spines of this region increase in antero-posterior extent from before backwards, the largest seen being about equal to the length of a centrum, 11 millims. ; they are as nearly as possible the

same height throughout, 10 millims., and each vertebra, with its centrum and spine, has a total height of 24 millims.   The pre- and post-zygapophyses are well shown. The transverse processes cannot all be seen, but by laying open some of them which were near the longitudinal break, we now know the form of the rib articulations of vertebræ Nos. 3, 5, 8, 11, and 13.   All the costal articulations being above the neuro-central suture, these vertebræ agree, in this respect, with the corresponding vertebræ of living crocodiles ; but they differ inasmuch as some of the anterior ones have distinct capitular and tubercular processes, while in crocodiles the corresponding thirteen presacral vertebræ have each a single long transverse process which carries both the costal articulations.   The most anterior vertebra, in the present specimen, has the tubercular process nearly as long as the neural spine (8 millims.), it is directed upwards and perhaps a little forwards, and its anterior and posterior edges are thin and continuous with the pre- and post-zygapophyses ; below and in front of it is seen the short capitular articulation quite at the front of the neural arch. Passing backwards, No. 11 vertebra has the tubercular process shorter (6 millims.) and directed outwards and a little backwards ; the capitular articulation is still distinct.   In No. 8 vertebra the two articular processes have united at their bases, and the tubercular process is shorter ; both articular surfaces may be said to be on the short transverse process.   No. 5 vertebra shows this character carried still further, and the transverse process is a little lower on the neural arch ; but there are still two articular surfaces.   In No. 3 vertebra the transverse process is as long as in No. 5, but narrower from before backwards, and, although the end still seems to be bifid, the two articulations are close together, if they do not join.

The Sacrum is composed of three vertebræ, the two hindermost of these are in their natural position, and seem to have been firmly united ; but the third has been pushed out of its place and somewhat broken.   Casts of the cavities from which the fragments of bone had been cleared away reproduce the forms of these vertebræ and their ribs (Plate 55, figs. 4, 5, 6).   The centra of the two united vertebræ have a total length of 23 millims., they are flattened inferiorily, and the terminal faces are flat.   The neural spines are about 10 millims. above the pre-zygapophyses and, with the centra, stand about 25 millims. high.   The middle spine of the sacrum is the broadest, having an antero-posterior extent of 13 millims.   The sacral ribs are strong quadrate processes, very similar to those of a crocodile, they are directed outwards and a little downwards, with expanded extremities, the hinder one being the largest and having its wide outer end oblique to the longitudinal axis of the vertebræ (fig. 4).   This large rib, it is tolerably evident, was attached to the posterior extremity of the ilium, which is but little out of place.

The anterior sacral vertebra has been troublesome to interpret, as it is not only pushed out of place but is partly broken, and the centrum is not shown.   At first it was uncertain whether this was part of the sacrum ; and the fact that the centrum was not fixed to the other two, as they were to each other, seemed to militate against

this interpretation, but a close examination has convinced me that it is correct. In the pre-sacral vertebræ, as we have seen, the transverse processes become smaller as we pass backwards, and are very small in the vertebra immediately in front of the one now in question, which retains, on the right side, a long and strong process (or rib) with an expanded end, quite as long as that of the vertebra next behind it although not so stout (Plate 55, fig. 4, *sa.* 1). The corresponding process of the left side has been separated from the vertebra, and is seen in the stone just above the ribs of the two hinder sacral vertebræ. The rib of this anterior sacral vertebra must have been in close relation to the broad anterior part of the ilium (see fig. 5), which would otherwise have been without support.

Caudal Vertebræ (Plate 54) to the number of twenty-one are preserved in a continuous series, and much of the bone is present, although in a very friable condition. The centra of the more anterior of the caudal vertebræ are very little shorter than those of the sacrum, being, as nearly as possible, equal to those of the thorax (11 millims.), and they decrease so little in length in the hinder part of the tail that the twentieth of the series measures 10 millims. The height of the centra, however, decreases more rapidly, for while the first caudal centrum is about 9 millims. high the twentieth is only 5 millims. In form also these centra resemble those of the thorax, but the inferior part is rounded and not keeled, and their articular faces are oblique to the long axes. Neural spines are exposed at both ends of the series, and also in the middle, the front one being about the same height and width as those of the sacrum (11 millims.); but while they gradually decrease in width they rapidly increase in length to the fifth, which stands 16 millims. above the zygapophyses, and with the centrum has a total height of 30 millims. Passing backwards from this point, the spines gradually shorten, the eleventh being 13 millims. and, with the centrum, 26 millims. high, while the twentieth spine is 8 millims. and, with the centrum, 15 millims. high.

The *chevron bones* are long and slender, and about sixteen of them are exposed; the anterior one is not less than 40 millims. long, and its proximal end is near the junction of the first and second caudal centra, but it may be somewhat out of place, for it is the third caudal centrum which first shows a distinct articular surface for a chevron bone, and this is at its hinder end. The transverse process of the first caudal projects about 10 millims. from the centrum, it is flattened and has an antero-posterior width of about 5 millims. at the base, but expands to 7 or 8 millims., and is rounded distally. The transverse processes of the next three vertebræ are somewhat longer and the width is maintained if not exceeded; beyond this they become narrower, and apparently shorter towards the end of the tail, but they are not clearly shown. All the transverse processes, so far as they can be traced, have a ridge on the under surface from base to apex, and a corresponding groove on the upper surface. On the first caudal vertebra these processes arise from the lower part of the neural arch and from the centrum, and project directly outwards; but in the succeeding vertebræ

they ascend the arch, so that on the sixth vertebra they are nearly on a level with the base of the neural spine. From this point backwards they remain practically in this position, but are directed more and more upwards.

Close under the transverse process of the anterior caudal vertebra there is a distinct tubercle on the side of the centrum, and a similar tubercle may be seen on the two or three succeeding vertebræ, but gradually getting lower down on the centrum. These tubercles are distinct on the left side, but less so on the right.

The Ribs are represented by a few slender bones seen below the thoracic vertebræ (Plate 56, fig. 1), but they are too imperfect to supply any satisfactory information ; close to the anterior vertebræ of this region, however, there are some fragments, one of which is clearly the proximal part of a rib with a distinct double head, such as would have articulated with the distinct capitular and tubercular processes of the thirteenth vertebra above described.

In front of the pubis and close to the edge of the block of stone (Plate 54) are a number of still more slender bones, which, from the manner of their meeting to form an acute angle, directed forwards, it is evident are abdominal ribs, but these also are too indefinite to allow very much of their structure or arrangement to be distinguished.

Pelvis.—The fragments which represented the bones of the pelvis and hind limbs were found to be insufficient to give any just idea of their form, and it was deemed best to clear them away and make casts from the cavities, as in other instances. The result has fully justified the attempt, the casts having reproduced the forms of the bones of the greater part of the pelvis, as well as those of the hind limb and foot, in greater detail than could have been anticipated.

The *ilium* (Plate 56, fig. 3) of the left side is shown, but it is thrown somewhat out of its natural relation to the other pelvic bones; and, as it is the ischium and pubis of the right side (Plate 56, fig. 4) which are best exposed, the precise mode of union of the three elements is not clear. The ilium is small compared with the other two bones ; its greatest length being 46 millims., while that of the ischium is 52 millims., and that of the pubis 70 millims. The ilium extends for about half its length behind the acetabulum, and is at this extremity acutely pointed; anteriorly it is obtusely angulated, slightly inflected above, and extends but little in front of the articular cup. The concave outer surface is definitely marked off from the acetabulum, which is a deep excavation bounded internally by bone ; but it is not certain whether it was completely closed or perforated. Both before and behind the acetabulum there is a short process, the ends of which were apparently articulated to the ischium and pubis ; and it seems almost certain that both these bones combined with the ilium to form the acetabular cup.

The *ischia* are both preserved; the inner side of the left one is seen in close relation with the left ilium (fig. 4), but it is partly hidden by the corresponding bone of the right side, which is the one best preserved, and shows the external surface ; its

greatest length is 52 millims.   The proximal end of the ischium is wide (22 millims.), its upper part being thickened to form an articular surface for the ilium and part of the articular cup, while the lower part is thin, forming a broad plate which unites with a similar plate of the pubis.   At about 18 millims. from the proximal end there is a distinct obturator process, behind which the bone is reduced to about 8 millims. in width and not more than 3 millims. in thickness.   The narrowest part of the bone (6 millims.) is at about the middle of its length.   Posteriorly the bone curves inwards and upwards, and widening again to about 8 or 9 millims., terminates by a rounded spatulate extremity.

The pubis (fig. 4) is 67 millims. long, it is broad proximally like the ischium, and has a similar width, with the upper part thickened for articulation with the ilium, and the lower part thin where it unites with the ischium.   The upper thickened part is proximally about 14 millims. wide; but this is rapidly reduced, its distal half being only about 3 or 4 millims. thick as seen from the side.   The inner parts of both pubes have been broken away by the splitting of the block of stone, but it seems probable that the distal half of each of them was flattened from above downwards.   A distinct ridge separates the proximal thickened part from the thin plate below; this ridge, at first directed downwards, gradually turns outwards, and becomes the external edge of the distal half of the bone.   The thin lower plate of this bone has a peculiar form; proximally where it evidently joined the corresponding plate of the ischium, it is directed downwards and a little inwards; passing forwards it widens, projects more inwards, and gets more towards the inner side of the thicker and upper part of the bone; this change continuing, the thin plate forms a scroll, which, at a distance of about 20 millims. from the proximal end, comes to be altogether on the inner side of the pubis, and seems to have been continued into a spatulate distal termination. In other words, as we trace the distal transversely flattened part of the pubis proximally, its inner part gradually turns downwards, so that its inner margin becomes the lower edge, where this plate joins the ischium.   To what extent the inner edges of the pubes met in the middle line cannot be ascertained.

Hind Limb.—Parts of both hind limbs are preserved (Plate 56, fig. 2), and it is evident that the skeleton, even to the small bones of the toes, was still connected by ligaments when it was buried in the Elgin sand.   The lower part of the right tibia and fibula, together with the right foot, are wanting, having been destroyed in the process of splitting open the block of stone.   Casts from the cavities in the stone have been prepared which reproduce all the parts of the limb now to be described. But it should be noticed that although the cast which has been figured (fig. 2) shows nearly all the bones described, the continuations of some of them, as well as a few bones not shown by this cast, are present on the opposing block of stone, and all these are represented in their proper position in this figure, by unshaded outlines.

The femur of the left side shows the form of the proximal end, and the full length of the bone; that of the right side has the distal half exposed.   In general form this

femur closely resembles that of the *Alligator Mississippiensis*, which I have for comparison ; it is, however, more slender, the trochanter is not so well marked, and the preaxial roughened protuberance is more prominent than in the alligator. The greatest length is 87 millims., the width of both extremities is the same, 16 millims. On the under surface of the fibular condyle (fig. 5) there is a small prominence, which may be altogether accidental, but, seeing that it occupies the position of the crest found in birds and Dinosaurs, which articulates between the tibia and fibula, it deserves to be noticed, as it may prove to be of morphological importance.

The *tibia* and *fibula* of the right side are only represented by pieces of their proximal ends, which are seen close to the right femur. The left tibia and fibula are also present, but they want their proximal ends. Although neither of these bones are complete, yet it is clear that they were but little, if at all, shorter than the femur. Both ends of these two bones are enlarged, much as they are in the alligator, but they are too imperfect to speak about very definitely.

The *tarsus* is represented by two bones, one of which is in contact with the distal end of the bone referred to as the left tibia, and is, doubtless, the astragalus ; it agrees better with the astragalus of a crocodile or lizard than with that of any Dinosaur. The second tarsal bone is merely a small ossicle which may have belonged to the distal row.

The *hind foot* of the left side is preserved in a most unexpected manner ; but that of the right side, as well as the greater part of the right tibia and fibula, is wanting, and the position and direction of the pieces of the latter which are preserved make it very unlikely that any of the right foot-bones are mixed with those of the left ; and further, the position and relations of the phalanges of each of the left toes show unmistakably that they belonged to one digit, and for the most part look as if they were still united by their decaying ligaments. There are five metatarsals, and these are numbered in the figure (Plate 56, fig. 2), in accordance with their gradually decreasing stoutness, but partly also on account of the number of phalanges in relation with them. Thus it will be seen that number 1 is the stoutest metatarsal, and number 5 the most slender. The second, third, and fourth metatarsals, however, are longer than the first or fifth. Metatarsal 1 has close to it two stout phalanges (*a* 1, *a* 2), the terminal one being ungual. Metatarsal 2 has three phalanges (*b* 1, *b* 2, *b* 3) close to its distal end, the last being ungual. Metatarsal 3 has no phalanges quite close to it, but it is evident from their size that the four rather smaller ones (*c* 1, *c* 2, *c* 3, *c* 4), which are close together, curled in a ring, belong to the third digit, the terminal phalanx of which was ungulate. Metatarsal 4 has near its extremity one phalange (*d* 1), while a little below this there are three small ones (*d* 2, *d* 3, *d* 4) in a series, and on the opposite block of stone, but continuing the series, is another very small one (*d* 5), making in all five phalanges to this fourth digit. In the cast figured metatarsal 5 has no phalanges near it, but, on the opposite block of stone, its counterpart is shown with two phalanges (*e* 1, *e* 2), in a

line with it, but somewhat separated, and these are indicated in the figure by unshaded outlines. The second phalange of this fifth digit has an articular surface at its distal extremity, and consequently there must have been at least one other phalange. This foot, it will be seen, agrees with that of modern lizards in having five digits, and also in the number of phalanges in the first four digits, and probably in the fifth also; namely, in the first 2 phalanges, in the second 3, in the third 4, in the fourth 5, and in the fifth certainly 3, and very likely 4 phalanges.

Scutes.—Upwards of forty scattered scutes may be counted above the vertebræ, between the two extremities of this specimen. The majority of these are more or less oval in outline, and several show signs of having been keeled. No definite markings can be seen on those of the caudal region, but two or three in the neighbourhood of the thorax and skull are ornamented with more or less distinct tubercles and radiating ridges (Plate 55, fig. 7). The oval outline of most of the scutes shows that they were not in close relation, but somewhat separated from each other; several, however, have one side straighter than the other, and this may indicate an arrangement in pairs although not united. In the front dorsal region there are several scutes having a more quadrate outline, and these may have been closer together; but there is no evidence of overlapping or of their having been definitely in contact with each other.

Affinities.

The pointed and beak-like extremity of the skull of this Elgin reptile, especially when seen from above, as well as the large pre-lachrymal fossas gives it a very bird-like appearance; but the similarity is only superficial, the details of its structure being more reptilian that avian. This skull has some resemblance to that of the Pterosaurian, *Scaphognathus*; both having a large pre-lachrymal fossa, and a similarly disposed jugal bone; the supra- and infra-temporal fossæ are likewise present in both, and the teeth are not very dissimilar; but in the form of the palate and other details the two differ widely, and still greater differences characterize other parts of the skeleton.

This Elgin fossil before it was fully freed from the matrix was referred to by Mr. Smith Woodward as a new genus of Aëtosaurian reptile, and the similarity, in many respects, between the two forms is obvious; but now that its structure is more clearly seen, important points of difference are observable, the significance of which will be best appreciated after a closer comparison. If the figures of *Aëtosaurus*, given by Dr. OSCAR FRAAS (56), be compared with ours (Plate 55, fig. 2) the two skulls will be found to agree in having paired frontals and parietals, as well as in the absence of a parietal foramen. In both also there are double and large anterior nasal openings, between which and the orbits are the large pre-lachrymal fossæ. The palate of *Aëtosaurus* is

not figured or described, which is the more to be regretted as it is an important point in the structure of these early forms of reptiles.   Dr. FRAAS however says (56, p. 13) "Die 3 Knochen, Zwischenkiefer, Nasenbein und Oberkiefer, umschliessen die vordere ovale Höhle, Nasenhöhle, welche nach unten offen zugleich das grosse Foramen incisium bildet," and this apparently indicates an anterior position for the primitive posterior nares, as in lizards.

Aëtosaurus differs from the present specimen in having broad parietals instead of narrow ones, with the supra-temporal fossæ pushed outwards quite to the sides of the skull.   Moreover the parietals do not, by a lateral process, bound the supra-temporal fossæ posteriorly.   In the Elgin skull there is a distinct and peculiar infra-temporal fossa, but nothing of the kind is shown in the restored figure of *Aëtosaurus* (56, p. 12).   If this restoration is correct, the absence of an infra-temporal fossa is a very distinctive feature ; but both the skulls figured by Dr. FRAAS on plates 2 and 3 seem to show a part of such a fossa, and if this be so, then the two forms will prove to be more nearly related than they now appear to be, judging from the restored figure ; and one of the chief characters excluding *Aëtosaurus* from the Parasuchia will vanish. The teeth of *Aëtosaurus* are uniform in size, with the crowns enlarged, somewhat as in *Stagonolepis* ; in our specimen the teeth vary much in size and their crowns are not enlarged.

The vertebræ of *Aëtosaurus* are said to be concave anteriorly and convex posteriorly, but only a few of them are seen, and those figured do not clearly show this procœlous character.   The pelvic bones are not alike in the two forms, which differ also in the number of the phalanges in each digit of the foot, so far as these can be made out.   Although the ornamentation of the scutes in the two animals is very similar, yet their arrangement differs widely, for while *Aëtosaurus* has the body completely encased in its closely locked armour, there is only a double row of dorsal scutes in the present specimen, and these are separated from each other, except perhaps in the front part of the body.

If *Aëtosaurus* should be found to agree with the present specimen in the possession of an infra-temporal fossa and bi-concave vertebræ, yet the other differences between the two forms would suffice to separate them at least generically ; but *Aëtosaurus* could hardly then be excluded from the Parasuchia.

The only forms from the British Trias, excepting the Parasuchia, which will be referred to later on, that need be noticed are the compressed and serrated teeth referred to *Teratosaurus* (= *Zanclodon*), *Cladyodon*, and *Palæosaurus* (60, 67) ; and even if the resemblance between these and the teeth of our specimen were greater than it is, one would scarcely be justified in referring the latter to any one of those genera, which have been founded upon teeth only, and all represent animals of much greater size.   *Zanclodon*, besides being much larger, has both edges of the tooth-crown serrated throughout, while the teeth of the other two genera although agreeing with those of the Elgin fossil in the restriction of the serration on the

anterior edge to its distal half, differ, the one in being much larger, and the other in having a much rounder section. However, the similarity between these teeth suggests the possibility of the Elgin fossil belonging to the same group of reptiles, and certainly it presents many points of resemblance to some of the Theropodous Dinosauria. The skull of *Megalosaurus*, if it has been correctly restored, and that of the American *Ceratosaurus*, notwithstanding their much greater size, seem to be constructed upon the same plan. (Compare Plate 55, fig. 1, with MARSH, 'Am. Journ. Sci.,' 1884, vol. 27, Plate 8, fig. 1.) There are similar supra-temporal fossæ and the four lateral apertures, with the bones arranged on the same plan ; the teeth are compressed and serrated, they also vary much in size, and the quadrate is similarly directed obliquely backwards. The palate of *Ceratosaurus* is not figured by Professor MARSH, and the description does not give the position of the posterior nares. Other parts of the skeleton of *Ceratosaurus* are less like the Elgin specimen than are those found in the much smaller Dinosaur *Anchisaurus,* more recently made known from the Trias of Connecticut (64), the skull of which is only slightly larger than that of our specimen, but although constructed upon essentially the same plan as that of *Ceratosaurus,* is less like the Elgin form inasmuch as the quadrate is set obliquely downwards and forwards, while the teeth are uniform in size, and of a different shape. The pelvis of the Elgin specimen makes, perhaps, a nearer approach to that of *Anchisaurus* than to that of any other Dinosaur ; but the ilium, having but little pre-acetabular extension, retains more of the crocodilian character ; while the ischium and pubis, in their large proportionate size, resemble those of *Anchisaurus.* The femur of the Elgin specimen, as we have seen, is crocodilian in form, and is not half the size proportionately of that of *Anchisaurus.* The astragalus of the latter genus is described as attached to the tibia in true Dinosaurian fashion, while in our specimen it is a separate bone and more like that of a crocodile or lizard.

The general resemblance which the skeleton of the little *Compsognathus* (79) bears to our specimen is very striking, especially as their position in the stone is so alike, and its affinity to the Dinosaurs above noticed leads one to expect similarities of structure. A close comparison, however, does not indicate a nearer relationship to our fossil than was found in *Anchisaurus.* The skull of *Compsognathus* is broken, but was probably formed on the same plan as that of our specimen ; the teeth are slender and conical, not compressed and serrated. The greater proportionate length which the tibia bears to the femur, and the hind limb bears to the rest of the skeleton, is unlike our fossil ; and then again the astragalus is said to be firmly attached to the tibia.

If we turn to the Parasuchia, we find that the obvious characters of our specimen prevent its reference to either of the known genera, namely, *Stagonolepis, Phytosaurus, Parasuchus,* and the above described *Erpetosuchus* ; yet it presents many of the characters which distinguish that group of reptiles. The skull has a large pre-lachrymal vacuity and distinct supra- and infra-temporal fossæ ; the primitive

posterior nares, if my interpretation of the palatal apertures be correct, are placed only a little further back than they are in *Phytosaurus*; the vertebral centra are slightly amphicœlous ; the ilium is of crocodilian type, although not so high as in *Stagonolepis*, and projecting a little more forwards, while the ischium and pubis are elongated bones approaching in form those of certain Dinosaurs. The limb bones seem to be of crocodilian type, and there is a row of dorsal scutes apparently in a double series. These characters, it will be seen, are essentially those laid down as characteristic of the Parasuchia by Professor HUXLEY, who, at the same time, pointed out (18, p. 41) how the "Parasuchia, in those respects in which they differ from the Mesosuchia, approach the Ornithoscelida and the Lacertilia" (especially Sphenodon). The many points of resemblance between the Parasuchia and certain of the forms usually included among the Dinosauria, have also been noticed by other writers ; and the difficulty of separating the two groups is increased by a study of this new Elgin reptile, which holds, as I think, a more intermediate position between the two series, than any form hitherto described, for although the characters of its skull and teeth find their nearest counterpart among the Dinosaurs, and the pelvis and limbs might belong to either a Theropodous Dinosaur, or a Parasuchian ; the form of the free astragalus is more Crocodilian than Dinosaurian. While acknowledging the difficulty of assigning this new reptile to either of these groups, it seems most in accordance with the facts to place it provisionally with the Dinosaurs.

Whatever doubt there may be as to the precise affinities of this most interesting reptile from the Elgin Sandstone, there will be none as to its being generically distinct from any known form, and I propose therefore to name it *Ornithosuchus Woodwardi*.

3. NOTE ON SOME FRAGMENTARY SPECIMENS.

Besides the specimens above described, I have received four others, all obtained from the quarry at Spynie, which, although too imperfect to allow of precise determination, should not be altogether passed over, more especially as the quarry, I am told, is now closed, and new specimens from this locality are not likely to be discovered for some time to come.

1. The first of these specimens was obtained by Dr. GORDON. It is contained in the two halves of a block of stone, which, when it reached me, weighed somewhat more than half a ton. Obliquely across the broken surfaces of this stone, for a distance of about twenty-four inches, a series of vertebræ could be traced, each about three-quarters of an inch long, while lying across these were many slender bones, evidently abdominal ribs, together with a few other bones, apparently dorsal ribs At each end of this series, portions of bones could be seen on the sides of the block, and it was hoped that with patience the limbs and skull might be displayed, but these hopes have not been realized. The bones have been much crushed and partially

dissolved, while they are, for the most part, so nearly the same colour as the matrix, and so intimately incorporated with it, that their forms could not be deciphered.   At one end, portions of the pelvis and of a femur may be seen; and, at the other, there is part of a skull, but these are so fragmentary and indistinct that I have been quite unable to satisfy myself as to the relationship of this fossil.

2. The second specimen was secured by Mr. H. H. HOWELL of the Geological Survey.   It is in several pieces, and comprises a few portions of vertebræ with centra less than three-quarters of an inch long, fragments of pelvic bones, and, extending outwards from these on each side, a femur which may have been six inches long.   A few slender bones are seen, which look like abdominal ribs.   This fossil may be part of a *Stagonolepis*, but there are no scutes to be seen.

3. The third specimen was likewise obtained by Mr. H. H. HOWELL.   It is part of a small reptile, the bones of which have been dissolved out; it shows part of the vertebral column, traces of the pelvis, and a very perfect hind limb, the bones of which were remarkably stout for their length.   The femur, tibia, astragalus, five metatarsals, and one or two phalanges are very nearly in their natural positions, but the fibula is somewhat displaced.   The cavities left by the vertebræ are too much broken to give a definite idea of their shape, but the deeply bi-concave character of their centra is well seen.   Four vertebræ together measure 25 millims.   The lengths of the other bones are: femur, 32 millims.; tibia, 22 millims.; metatarsals, 1 to 5, gradually increasing from 8 to 10 millims.   This specimen must have been smaller than the *Erpetosuchus Granti*, from which it differs in having deeply bi-concave vertebræ; moreover, its short and stout hind limb would scarcely accord with a long and slender fore limb, such as that of *Erpetosuchus Granti*.   The specimen probably represents a lizard of about the size of the living *Sphenodon*.   It is much larger than *Telerpeton Elginense*, but may, perhaps, belong to another species of that genus.

4. The fourth specimen, sent to me by Mr. TAYLOR, of Elgin, is interesting as having been found at Spynie only a short time before the closing of the quarry; but it is in several disjointed pieces, and the bones are scattered, the most perfect part being a row of neural spines, which agree in size with, and are not unlike those of *Erpetosuchus Granti*, but it would be very hazardous to refer these remains to that genus.

III. LIST OF WORKS CONSULTED.

The reference numbers follow on from those given in the earlier paper (68), and any less number than 50 mentioned in the present paper refers to the previous list.

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IV. EXPLANATION OF PLATES.

PLATE 53.

Erpetosuchus Granti, gen. et sp. nov.

All the figures, except 7 and 8, are natural size, and have been drawn from gutta-percha casts taken from the cavities in a block of Elgin Sandstone, in the possession of Mr. JAMES GRANT of Lossiemouth.   The exact locality of the specimen is uncertain.

Fig. 1. Skull and lower jaw seen from the right side. The anterior part of the right ramus being still hidden by the matrix, it has been completed by reversing the left side, fig. 4.

Fig. 2. Skull seen from above ; a portion of the left side restored in outline.

Fig. 3. Skull seen from below.

Fig. 4. Lower jaw, left ramus, articular end hidden in matrix.

Fig. 5. Series of vertebræ and scutes immediately behind the skull, seen from left side.

Fig. 6. Part of same series seen from right side.

Fig. 7. Vertebra No. 9, enlarged and restored.

Fig. 8. Four scutes enlarged.

Fig. 9. Right scapula and coracoid seen from outside.

Fig. 9A. Same seen from inner side.

Fig. 10. Left humerus, under surface.

Fig. 10A. Same bone, front view.

Fig. 11. Left tibia and fibula seen from behind, with under surface of five meta-carpals and some phalanges.

Fig. 12. Right humerus, upper surface.

Fig. 13. Right tibia and fibula seen from before, with upper surface of five meta-carpals.

Fig. 14. Interclavicle, upper surface.

PLATE 54.

Ornithosuchus Woodwardi, gen. et sp. nov.

From a photograph about one-third natural size, by Messrs. WALKER and BOUTALL, of a specimen obtained by Dr. GORDON from the Elgin Sandstone of Spynie, to be preserved in the British Museum.   The under surface of the skull with the lower jaw in place is seen on the left ; the dorsal and caudal vertebræ extend obliquely across the stone ; below them are the pelvis and limb bones, and above them are the

scattered scutes. At the lower part of the slab and towards the left the group of abdominal ribs are seen.

PLATE 55.

Ornithosuchus Woodwardi.

Same specimen as Plate 54. All the figures except 1A are drawn natural size.

Fig. 1.   Skull and lower jaw, right side. Much of the front parts of the right side being hidden in the specimen, it has been completed in the figure from the left side, and the right ramus has been similarly restored by reference to both rami.

Fig. 1A. The largest tooth, twice its natural size.

Fig. 2.   Skull from above.

Fig. 3.   Skull from below.

The following figures are drawn from gutta-percha casts.

Fig. 4.   Vertebræ seen from right side—5 caudal, 3 sacral, and 1 pre-sacral. The sacral ribs have been completed from the left side. This pre-sacral vertebra is shown also in the series on Plate 56, fig. 1.

Fig. 5.   Sacrum and left ilium seen from above.

Fig. 6.   Sacrum seen from below.

Fig. 7.   One of the largest and most perfect scutes.

PLATE 56.

Ornithosuchus Woodwardi.

Same specimen as Plate 54. All the figures natural size, and drawn from gutta-percha casts.

Fig. 1.   Series of pre-sacral vertebræ seen from the right side. The vertebræ are each numbered from the sacrum forwards. No. 1 vertebra is also shown in the series on Plate 55, in order that the relations of the two series may be understood.

Fig. 2.   Parts of pelvis and limb bones seen from the left side. The unshaded outlines completing several parts are supplied from the opposite slab of stone. The various bones are indicated by letters, the right and left

sides being marked respectively *rt.* and *lt.*   All the foot bones belong to the left side—*ast.*, astragalus ; *mt.*, 1, 2, 3, 4, 5, metatarsals ; *a*, 1, 2, phalanges of first digit ; *b*, 1, 2, 3, phalanges of second digit ; *c*, 1, 2, 3, 4, phalanges of third digit ; *d*, 1, 2, 3, 4, 5, phalanges of fourth digit ; *e*, 1, 2, phalanges of fifth digit.

Fig. 3.   Left ilium, outer surface, nearly complete.

Fig. 4.   Right pubis with right and left ischia seen from right side.   The lower edge of left acetabulum is also seen.

Fig. 5.   Right femur, under surface of distal half.

Fig. 6.   Left femur, under surface of proximal half.

LETTERING USED IN THE FIGURES.

a, b, c, d, e. Phalanges of digits.

ang. Angulare.

art. Articulare.

ast. Astragalus.

bo. Basioccipital.

bs. Basisphenoid.

den. Dentary.

exo. Exoccipital.

fem. Femur.

fib. Fibula.

fr. Frontal.

il. Ilium.

is. Ischium.

i.tem. Infra-temporal fossa.

ju. Jugal.

la. Lachrymal.

lt. Left.

mt. Metatarsal.

mx. Maxilla.

n. Anterior narial aperture.

na. Nasal bone.

op.ot. Opisthotic.

orb. Orbit.

p. Pubis.

pa. Parietal

p.la. Pre-lachrymal fossa.

pmx. Premaxilla.

pr.fr. Prefrontal.

p.sa. Pre-sacral vertebra.

pt. Pterygoid.

p.tem. Post-temporal fossa.

pt.fr. Post-frontal.

pt.na. Posterior narial aperture.

pt.o. Post-orbital.

pt.pl. Post-palatine vacuity.

qu. Quadrate.

qu.ju. Quadratojugal.

rt. Right.

s.a. Sacral vertebra.

s.ang. Surangular.

sq. Squamosal.

s.tem. Supra-temporal fossa.

tib. Tibia.

tr. Transverse bone of palate.

t. Plate 55, fig. 2, tooth of lower jaw.

Fig 6'
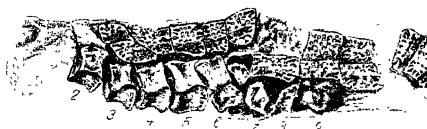
Fig 5

Fig 8
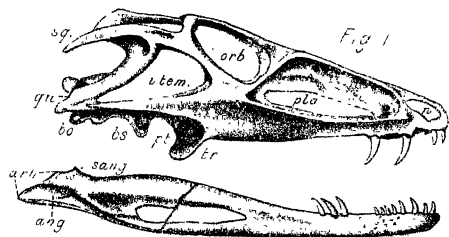
Fig 1
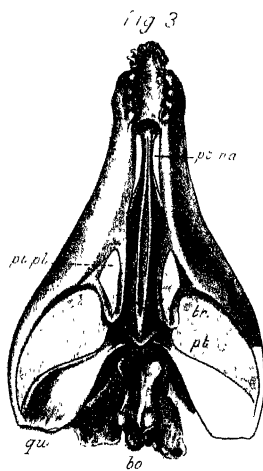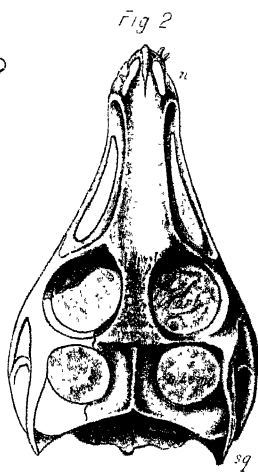
sq. *orb*

qu. *i tem.* *pla*

bo *bs* *ft* *tr*

art. *sang*

ang

Fig 7

Fig 9

Fig 3

Fig 14

Fig 2

Fig 9e
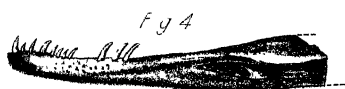
pt na

pt pl.

tr

pt

qu

bo

n

v cer

sq

Fig 10

Fig 12

Fig 11

Fig 4

Fig 13

ORNITHOSUCHUS WOODWARDI.   GEN. ET SP. NOV.

Fig 4

sa 1

sa 3   sa 2

p sa 1

5   4   3   2   1

pt fr   pr fr

sq   pt o   la   Fig 1   na

orb

p la   n

ι tem   pmx

qu

qu ju   ju   mx

Fig

7

pmx   Fig 2   pmx   Fig 3

t θ   den

a   mx

Fig 5   la

orb   1   pt p

2   pt na   tr

Pa   s tem   3

Fig 6

1

2   pt qu

3

Fig. 1

Fig. 3

lt il lt is rt is lt fem rt fem

Fig. 4

Fig. 5

rt. p

ast
mt 1
lt tib
t
mt 5
lt fib
a 1
a 2
e 1
mt 4
b 1
d 1
rt fib
rt f
mt 3
mt 2
e 2
c 1
b 2
o 2
Fig 6
d 4
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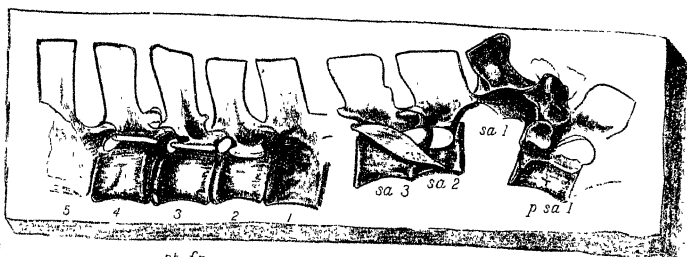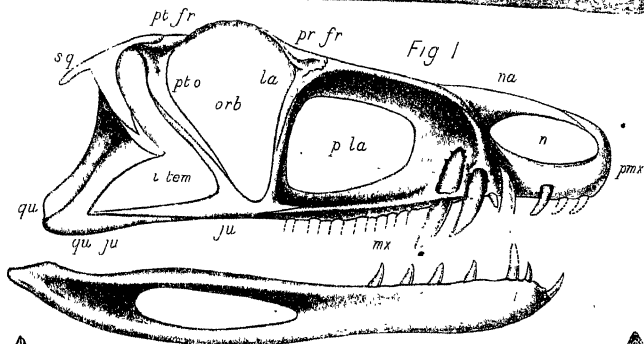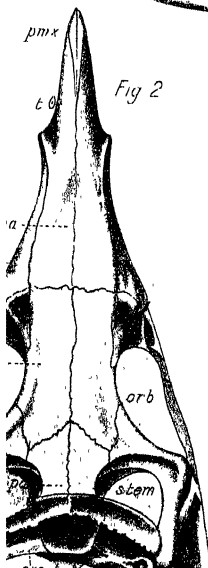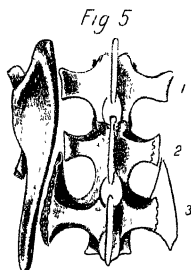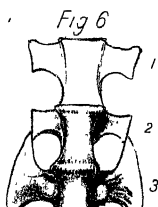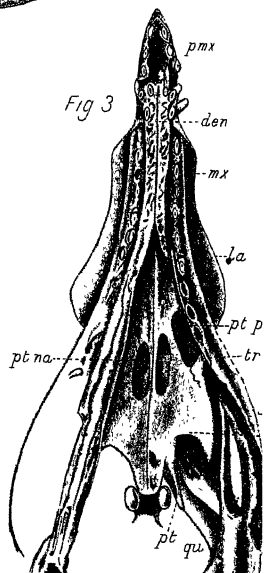
XIV. *The Effect produced upon Respiration by Faradic Excitation of the Cerebrum in the Monkey, Dog, Cat, and Rabbit.*

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[PLATES 57–59.]

CONTENTS.

INTRODUCTION.

The object of the following investigation was to elucidate the character of the representation of respiration in the highest nerve centres.   For this purpose the excitation method was employed, so that the results of the research are embodied in the effects produced on the movements of respiration when various regions of the cerebral hemisphere are excited.

By a careful regulation of the anæsthetic state in the species of animal used, by a recognition of the exact spot excited, and by employing a suitable stimulus, a constant effect upon respiration can be obtained from the cerebral cortex according to the point stimulated.   And the same result can also be obtained along a line joining the area discovered in the cortex with the medulla oblongata.

It is absolutely necessary, in order to get constant results, that the degree of anæsthesia employed should be accurately recognized.   This has apparently not been done before.   While some previous observers have, in fact, not used an anæsthetic at all, others have employed considerable doses of morphine or chloral.   Experiments by different observers on non-anæsthetized animals have yielded variable results, due to the fact that other disturbing factors have not been taken into account.   One of these is apnœa; for instance, any excitation of a sensitive part may cause acceleration of the respiratory rhythm, and, as a consequence of this acceleration, slowing, or even a short arrest.   During the prevalence of the apnœa the excitability of the brain is much lowered, so that the same stimulus re-applied to the same spot produces a different change in the respiratory rhythm.   Other conditions produce or add to the anæsthetic state, such as loss of blood, exposure of the brain, extravasated blood

pressing on the brain, general exhaustion of the animal, or some disease present in the animal. On the other hand, morphine or chloral in considerable doses completely obliterates some of the respiratory effects.

In the four species of animals, monkey, dog, cat, and rabbit, it is easy to form a judgment as to the state of anæsthesia—at least, after paying a little attention to the point. The rapidity of the corneal reflex may act as a guide, or, better still, the excitability of the second division of the 5th nerve in the orbit, or of the dura mater.

GENERAL EXPERIMENTAL METHOD.

The essential preliminary to every experiment has been a regular respiratory rhythm, obtained by employing such a stage of anæsthesia as proved by experience to be best suited for the particular experiment proposed. A tracing of the respiration and circulation before, during, and after the application of the electrical stimulus was taken in each experiment, and from the tracings alone have the conclusions furnished been drawn.

All the experiments have been repeated many times in each species of animal. Although, of course, only comparatively few tracings can be included in this paper, there is no point mentioned of which I have not many illustrations amongst the tracings in my possession.

The Age of the Animals.—In accordance with what has been known for many years (LEGALLOIS,* SEMON and HORSLEY,† &c.), the age of the animal is an important feature in the innervation of respiration. I have, therefore, in all cases used adults only.

Record of the Respiratory Movements.— After anæsthetizing the animal a tube was inserted into the trachea, so as to exclude any mechanical obstruction to the respiration, or any complication of the result by a simultaneous excitation of the larynx, whilst, at the same time, by this means the anæsthetic can be more readily regulated. A PAUL BERT's transmission apparatus, connected with a MAREY's receiving tambour, was fixed by an inextensile band round the lower third of the thorax, where the circumference enlarges most during ordinary respiration. The tracings, therefore, in the present research record the variations in the circumference of the lower third of the chest. In all cases the tracings are to be read from left to right, the upstroke representing the enlargement in inspiration of the chest from the position of equilibrium, and the downstroke the return during expiration to the position of rest.

The following terms applied to the respiratory tracing will be made use of in order to distinguish between the very different degrees of respiratory movement observed.

* LEGALLOIS, ' Œuvres.' Paris, 1830. Vol. 1, 1.

† SEMON and HORSLEY, " The Central Motor Innervation of the Larynx." ' Philosophical Transactions,' 1890, B, p. 187.

Expiration.—The line drawn through the lowest points of the normal tracing.

Active Expiration.—Any line below expiration.

Inspiration.—A line drawn through the tops of the normal respiratory curve.

Over-inspiration.—Any line above inspiration.

Record of the Circulation.—The cannula was placed in the carotid of the dog, cat, and rabbit, and in the common iliac in the case of the monkey, and connected with a mercury manometer.

Although this was regularly done, I propose not to refer at length to the changes in the circulation obtained simultaneously with the respiratory ones, but to reserve my observations under this heading for a future communication.   I have noted, however, on page 653, two well marked effects which may be found.

The Stimulus.—This was obtained through a DU BOIS-REYMOND Inductorium from two GRENET's cells.   The current was just sufficiently strong to be perceived by the tip of the tongue when the secondary coil was 12 centims. from covering the primary. The numbers 12, 10, 8, 6, 4, 2, 0 will be used as abbreviations to express the strength of the faradic stimulus when the secondary coil was 12, 10, 8, 6, 4, 2, 0 centims. distant from covering the primary.

The electrodes were of platinum, about 1 millim. apart.

An electromagnet in the primary circuit served to indicate the application and duration of the stimulus.

The rate of movement was marked by a metronome beating seconds, and recording by an electromagnet.

The recording points of respiration, circulation, and stimulus were kept vertically in line.

The recording apparatus used was the Kymographion of HÜRTHLE.

The Course of an Experiment.—The animal being suitably anæsthetized, and the point to be stimulated exposed and gently dried, the electrodes were placed against the surface of the brain.   A tracing was first taken with the electrodes in position before the stimulus, secondly, during the excitation, and thirdly, it was continued after the cessation of the stimulus until the respiration returned to its previous condition.

A current less than sufficient to produce any effect was first employed and then one of increased strength.   The stimulus was applied for six seconds or more, and was often repeated at the same spot at short intervals.

Exposure of the Brain.—The skull was always removed so as to sufficiently expose the part to be excited, the bleeding being stopped by soft wax and amadou.   The dura mater was raised as occasion required, and the brain kept warm and moist throughout the experiment by salt solution at blood heat, save that the spot was dried where the electrodes were to be applied.

The contents of the orbit were removed, or that of the eyeball only and the eyebrow drawn forwards.

In hemi-sections or in sections of both hemispheres, the plane of the surface was made perpendicular to that of the under-surface of the brain at the point of section, and also at right angles to the longitudinal axis.   In hemisections, particular care was taken to reach up to the middle line and not beyond.

A certain amount of hæmorrhage inevitably ensued in making such hemisections, but it was controlled by pressing the vessels against the base of the skull with sponges and amadou.

HISTORICAL RETROSPECT.

Alterations in respiration have been frequently noticed by various investigators during experiments upon the cerebral cortex and upon the basal ganglia, but the results obtained have not agreed together, or have been negative.

I am disposed to think that the variations in the results have been due to the use of non-anæsthetized animals in which the effects upon the respiration were complicated by other sensorimotor effects.   The negative results followed from not stimulating excitable spots, or because the animal had been too deeply anæsthetized by drugs or in other ways.

DANILEWSKY[*] used young cats and dogs slightly morphinized, and recorded the respiration by a tracheal cannula.   He obtained two kinds of results.   Cortical excitation in the region of the facial centre of HITZIG produced at first a slowing of the respiration with increased amplitude.   With a stronger stimulus a deep inspiration was followed by a longer expiration and a pause.   These phenomena only occurred sometimes, and often took place after the stimulus had ceased, even as much as 10 seconds after in one case.   A sufficiently strong excitation applied towards the base of the brain, especially when it affected the cerebral peduncles, caused marked acceleration of the respiration similar to the result of stimulating the dura mater and the peripheral nerves.   The arrest of respiration in these experiments may, from my observations, have possibly been due to the morphine, for slowing of respiration is observed to occur without any stimulus in dogs and cats well under the influence of morphine, and arrest may be produced in such animals by the excitation of any sensory part.

LÉPINE[†] laid stress upon the increased frequency of the respiratory movements.

BOCHEFONTAINE[‡] produced rapid movements of inspiration, followed by spasms of varying intensity, and finally convulsions.   The experiments were made on non-anæsthetized animals.

RICHET[§] employed dogs so deeply chloralized that the limbs did not react to cortical

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     * DANILEWSKY, "Experimentelle Beiträge zur Physiologie des Gehirnes." 'Archiv für Physiologie, Pflüger,' 1875, vol. 11, p. 128, see Table VI. and VII.

     † LÉPINE—quoted by FRANÇOIS FRANCK, see below.

     ‡ BOCHEFONTAINE, 'Archives de Physiologie,' BROWN-SÉQUARD,' 1876, vol. 3, series 2, p. 168.

     § RICHET—quoted by FRANÇOIS FRANCK, see below.

excitations. He then found that stimulation of the sigmoid gyrus arrested respiration. The same result occurred not only at many other points of the cortex, but also was obtained from the sciatic nerve.

Both chloral and morphine, according to my own observation, tend to produce an irregular rhythm in which pauses occur at uncertain intervals. This change may sometimes be induced by any sensory stimulus.

MUNK's* observations in this respect were published in 1883, and reprinted in 1890. Other experimenters have not confirmed them. When the electrodes were placed upon the frontal lobe, some millims. in front of the frontal, i.e., the crucial sulcus, and somewhat lateral to its median end (just where a shallow longitudinal depression runs from before backwards), respiration was arrested with the secondary coil at 7 or 6. The arrest was in deepest inspiration, and the diaphragm in extreme tetanic contraction. Very often acceleration of the respiration preceded the inspiratory tetanus, during which, by deeper inspiration and less expirations, the thorax and diaphragm came to occupy gradually the maximal inspiratory position, and were finally fixed in that position. The abdominal muscles during this time remained relaxed. When the electrodes were placed on the under-surface of the frontal lobe, about the middle, there followed from stimulus 7 or 6 either a powerful tetanus of the abdominal muscles in a maximal expiratory phase, or the latter contracted with great frequency but only to a very small extent, and returned with short jerks to the position of rest. If the electrodes approached the bulbus or tractus olfactorius sneezing or cough was produced.

In the ape along the horizontal limb of the præcentral sulcus, and between it and the middle line, with the stimulus at 7 or 6, the thorax and diaphragm assumed an inspiratory position; further outwards than this fissure excitation produced a tetanus of the abdominal muscles.

As far as they go these observations are in the main confirmed by my own.

FRANÇOIS FRANCK† made a further examination of the subject and criticized the work of previous observers. He pointed out that the arrest of respiration produced by excitation of the cortex was frequently due to apnœa following accelerated respiration or was produced by the severe tetanic convulsions into which the animal was thrown. He further showed that under deep morphine or ether respiration frequently became irregular with pauses which were very likely to coincide with an electrical stimulus. He concluded that excitation of the motor area with a sufficiently strong and prolonged stimulus produces modifications in the respiratory movements, and that excitation of other parts of the cortex does not produce respiratory reactions except in the case of convulsions, i.e., when the effect of the stimulus spreads to the motor area or tracts. The respiratory rate quickens or slows without any correspondence between the spot stimulated and the effect produced. It is according to him rather

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* MUNK, 'Ueber die Formationen der Grosshirnrinde,' Berlin, 1890, p. 164.

† FRANÇOIS FRANCK, 'Leçons sur les Fonctions motrices du Cerveau,' 1887.

the degree of intensity of the excitation or the degree of excitability of the cerebrum which appears to influence the frequency. The strongest stimulus produced slowing and exceptionally arrest, so that one cannot discuss centres for acceleration or for slowing.

A change in amplitude, augmentation, or diminution may be observed both with an increased and a decreased rate. The inspiratory state coincides with the greater amplitude and the expiratory state with the less. Special points for inspiration and expiration cannot be found upon the cortex, each of the points of the motor zone can produce the respiratory modifications indicated above.

UNVERRICHT* experimented upon dogs anæsthetised with morphine and recorded the respiration by means of an œsophageal cannula. With a faradic current less than sufficient to produce muscular contractions when applied to the 'motor' area, he obtained in some of the experiments slowing of the respiration by excitation of an area on the third external convolution outside the orbicularis centre.

PREOBRASCHENSKY† experimented with dogs under morphine and with cats under chloroform and ether. One-third of the experiments were negative. In the dog he obtained arrest of respiration for two or three seconds immediately after opening the skull, using a very weak faradic current, but he never saw an active contraction of the respiratory muscles. In some cats he obtained arrest in inspiration, in others no effect. His two last experiments were negative. The area stimulated was the same as that investigated by UNVERRICHT, viz., on the anterior part of the third external convolution.

It is well known that centres influencing respiration have been inferred from excitation experiments upon the cerebrum, and have been called "Cerebral Respiratory Centres."

CHRISTIANI,‡ experimenting upon rabbits, concluded that an inspiratory centre was situated in the lateral wall of the third ventricle, in the optic thalamus, just in front of the corpora quadrigemina and the commencement of the aqueduct. The area was extremely limited, only 1 millim. square, from which an electrical, mechanical, or thermal stimulation evoked an arrest of the diaphragm in inspiration, or a remarkable acceleration of the respiratory rate, together with an increase in amplitude. The centre was symmetrical on each side. Excitation of the corpora quadrigemina, just below or to one side of the aqueduct of SYLVIUS, caused an arrest in expiration. These latter experiments were made after the removal of the hemispheres, corpora striata, and optic thalami by vertical transverse section through the commencement of the aqueduct. Thus the 'inspiratory' centre had been removed before the 'expiratory' one was excited.

* UNVERRICHT, "Ueber die Innervation der Athembewegungen," 'Kongress für innere Medicin,' 1888, vol. 7, p. 237.

† PREOBRASCHENSKY, "Ueber Athmungscentren in der Hirnrinde," 'Wiener klinische Wochenschrift,' 1890, p. 833.

‡ CHRISTIANI, 'Zur Physiologie des Gehirnes.' Berlin, 1885, chapter 1.

MARTIN and BOOKER* stimulated the corpora quadrigemina in rabbits by plunging the electrodes into the substance of the mesencephalon. When the points were near the iter, acceleration was produced, which was changed into inspiratory arrest on applying a strong stimulus. By plunging the electrodes deep into ·the pons they obtained arrest in expiration. They experimented on two cats only and obtained the same inspiratory results as in the case of the rabbit.

The last-named observers, by plunging in the electrodes, appear to have stimulated the same points as myself on the surface of vertical transverse sections. Their experimental results varied, perhaps because they did not suitably anæsthetize their animals. CHRISTIANI, no doubt, stimulated in the line of the tract which I shall later on describe as causing acceleration and over-inspiratory tonus. Again, the shock to the rabbit of making a vertical transverse section of the brain through the beginning of the aqueduct, renders the animal deeply anæsthetized. CHRISTIANI, under these circumstances, obtained arrest of respiration probably in the line of the tract of fibres to which I shall refer as causing arrest when stimulated.

In view of the importance of this subject, I have repeated their experiments in the way the observers aforenamed performed them, and have obtained the same results. But I fail to see why the presence of "respiratory centres" should have been assumed from these experiments, if the term "respiratory centre" be used in anything like the same sense as when applied to the representation of respiration in the floor of the 4th ventricle. In fact, excitation experiments of this kind give results which are equally well or even better attributed to the results of stimulating fibres than of stimulating central mechanisms.

KNOLL,† experimenting more recently, does not say anything about the use of an anæsthetic. He found that excitation of the optic thalamus and corpora quadrigemina caused quickening of the respiration. A strong current applied to the under-surface of the anterior corpora quadrigemina caused spasm, especially of the eyes, tail, and flanks, the respiration becoming shallower or arrested. He never obtained an expiratory arrest. He concluded, from his experiments, that there was no respiratory centre in either the optic thalamus or in the corpora quadrigemina, but that the effect of excitation of these regions was to produce impulses in psychical or sensory tracts leading to true respiratory centres in the medulla and cord.

. His experimental results and the conclusions drawn seem to me to follow from using non-anæsthetized animals.

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* MARTIN and BOOKER, 'Journal of Physiology,' vol. 1, p. 370.

† KNOLL, 'Sitzungsberichte der Akademie der Wissenschaften,' Wien, 1885, vol. 92, sec. 3, p. 328.

DESCRIPTION OF PHOTOGRAPHS

On mature consideration, I think it best to give a description of the photographs first of all, in order that the position of the points stimulated may be the more easily understood.

On the photographs of brains and sections of brains have been indicated by marks the points where the several effects to be afterwards described in detail were obtained.

Rabbit's Brain (see Plate 57).

Photograph I*a*. A photograph of the under-surface of a rabbit's brain.

A ring has been placed upon the strip of cortex between the outer margin of the olfactory tract and the rhinal fissure just in front of the point where the sylvian artery crosses the fissure. The ring marks the point which will be referred to as the spot, on the application of the stimulus to which respiration can be arrested. It also shows the junction of the olfactory bulb and tract, marked by a square, the point from which over-inspiratory clonus was obtained.

Photograph I*b*. A photograph of the upper surface of a rabbit's brain, slightly enlarged.

The cross marks the point at which a branch from the anterior cerebral artery curves over from the mesial to the convex aspect. This is the place where the most marked acceleration can be obtained by the faradic stimulus.

Cat's Brain (see Plate 57).

Photograph II. A photograph of the antero-inferior and outer aspect.

A small ring is placed upon the apex of the olfactory lobe, *i.e.*, the antero-internal end of the triangular convolution formed by the outer border of the olfactory tract, the rhinal fissure and the furrow made by the sylvian artery. This is the place where arrest can be obtained.

An outer line delimits a semicircular area, at the border of which respiration could only be arrested by the strongest current used. The border line commences at the junction of the olfactory lobe and tract, crosses the supraorbital fissure some 3 to 4 millims. from its lower end and also the anterior composite lobe without extending as far as the anterior end of the coronary fissure. The line touches the anterior end of the anterior suprasylvian fissure, runs backwards parallel to and from 2 to 3 millims. above the rhinal fissure, nearly to the anterior end of the anterior ectosylvian fissure. The limit then turns downwards and joins the rhinal fissure about the point where the sylvian artery crosses. The outer part of the olfactory tract forms the lower border of the area.

The upper and inner end of the supraorbital fissure just beyond the limit of the photograph is the spot where acceleration is most marked.

The junction of the olfactory bulb and tract is also shown.

*Dog's Brain** (see Plate 57).

Photograph III. A photograph of a dog's brain in the same position as that of the cat's.

A small ring has been drawn upon the olfactory lobe between the junction of the supraorbital with the rhinal fissures in front, and the sylvian artery behind, to indicate the centre of the area by the excitation of which arrest of respiration can be produced. An outer line marks the limit where respiration could be arrested only by the strongest current used. This line commences on the olfactory lobe about 4 millims. in front of the junction of the supraorbital and rhinal fissures, it crosses the rhinal fissure about the point where there is a small notch to be seen, and the orbital lobe, to the supraorbital fissure, some 4 millims. from its lower end. Behind this point it passes backwards and downwards to the point where the sylvian artery traverses the olfactory lobe and rhinal fissure. The outer part of the olfactory tract is the lower border.

The upper and inner end of the supraorbital fissure is just beyond the photograph ; it is the point where the faradic stimulus causes the most acceleration.

The junction of the olfactory bulb and tract is shown.

Monkey's Brain (see Plate 58).

Photograph IV. A photograph of the under-surface of a monkey's brain.

A small semicircle placed just external to the olfactory tract in front of the sylvian fissure indicates the centre of the area for respiratory arrest. A larger semicircle marks the limit within which arrest could be obtained with the strongest current used. Commencing at the outer border of the olfactory tract about the middle of its length, the limit passes directly outwards to the transverse portion of the H-like orbital fissure, thence along the edge of the shallow concavity which the orbital lobe forms, across the sylvian fissure, and turning inwards on the temporo-sphenoidal lobe reaches the outer side of the optic commissure, some 2 millims. or so behind the sylvian fissure.

PHOTOGRAPHS OF VERTICAL SECTIONS OF THE CEREBRUM.

Upon the photographs have been placed the following marks :—

A Small Ring.—This indicates the point on each half of the section, by the stimulation of which arrest was obtained.

A Cross.—This indicates the point on each half of the section, by the stimulation of which marked acceleration was caused.

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* For the names applied to the cat's and dog's brain, see LANGLEY, "The Structure of the Dog's Brain," 'Journal of Physiology,' 1883–1884, vol. 4, p. 248.

A Square.—This indicates the point on each half of the section, by the stimulation of which over-inspiratory clonus was produced.

The Letter I.—This indicates the point on each half of the section, by the stimulation of which well-marked over-inspiratory tonus and tetanus was most easily obtained.

Vertical Sections of Cat's Cerebrum (see Plates 58 and 59).

Photographs V. to XV. A series of photographs, taken from Dr. HOWARD TOOTH'S microscopical preparations of a cat's brain, upon which marks have been made as above.

During the course of the research, I made use of a series of photographs taken from Dr. HOWARD TOOTH'S microscopical preparation of a monkey's brain, and also photographs made from sections of the brain of the rabbit and dog. The points were mapped in on these photographs, but, on account of the similarity shown to the results obtained in the cat, these photographs have not been reproduced.

THE DETAILS OF THE EFFECTS OBTAINED BY EXCITATION OF THE CORTEX CEREBRI AND OF THE SURFACES OF VERTICAL TRANSVERSE HEMISECTIONS OR SECTIONS MADE THROUGH THE CEREBRUM.

A. DIMINUTION OF ACTION.

(*a.*) *Slowing and Arrest of the Respiration obtained from the Cerebral Cortex.*

The Necessary State of Anæsthesia in Experiments upon the Cortex.—In order to obtain arrest of respiration by excitation of the area shown in the photographs, the animal must be narcotized so that no voluntary or reflex movements are evoked by the excitation. But a slight corneal reflex must still exist, and a strong stimulus still be able to disturb the respiration through the dura mater, fifth, or sciatic nerves. When these effects have been abolished, the animal is too deeply anæsthetized, and the cortex will be found no longer excitable to the strongest current used. But the excitability quickly returns if the animal be allowed to take a few breaths of air free from ether.

It is necessary to use ether. Morphine, except in very small doses, tends to abolish the excitability of the cortex, to render the respiratory rhythm irregular, and, moreover, any excessive dose is not so quickly got rid of as ether.

The cat may easily be maintained in the suitable stage of anæsthesia, especially when the experiment is confined to the arrest area, so that it is not awakened by excitation of the sensori-motor region, nor on excitation of the dura mater.

The rabbit presents somewhat greater difficulties, especially as there is only one very limited point on the cortex whence arrest of respiration can be evoked.

Dogs and monkeys are not so easily maintained in the same state as the cat, they

are apt to quickly pass into a too superficial or a too deep anæsthesia. But if an extremely small dose of morphine be injected subcutaneously half an hour before the commencement of the experiment, the animals can be kept in a more constant state of anæsthesia, resembling the condition produced in the cat by ether alone. The dose of morphine in this case must not exceed 0·01 gr. per 1 lb. (0·0014 grm. per 1 kilo.) live weight, injected subcutaneously, *i.e.*, 1 minim per 1 lb. live weight of the 1 per cent. morphine hydrochlorate solution.

The rabbit however cannot be given morphine even in very small doses without depressing the excitability of the cortex so that no arrest can be obtained. In my first experiments I found the arrest area in the dog and monkey without morphine and I have not used any morphine at all in the cat.

The Necessary State of Anæsthesia in Experiments upon Vertical Hemisections or Sections.—The deepest ether anæsthesia in which the animal will breathe regularly is required when exciting the surface of sections in order to obtain arrest and to obliterate all the effects of the stimulus upon other fibres, *e.g.*, those sensori-motor and commissural ones which increase the activity of the respiratory movements. Rabbits are the most difficult to get to breathe regularly in deep ether anæsthesia. Here also a minute dose of morphine, 0·01 grm. per 1 lb. (0·0014 grm. per 1 kilo.) live weight, may be tried in such animals as do not breathe freely in deep ether anæsthesia. The morphine aids in keeping the animal deeply anæsthetized with a smaller quantity of ether.

Slowing and Arrest of Respiration by Excitation of the Cortex Cerebri of the Cat.
  See Photograph II.

The Strength of the Faradic Stimulus.—At the centre of the arrest area the stimulus required to arrest respiration was generally 7, or 6, sometimes 8. Weaker currents were not quite strong enough to stop respiration but caused slowing. Currents weaker still had no effect. The further away the point stimulated was from the centre of the area, the stronger the stimulus required until the limit was reached where 0 was necessary to check the breathing. Beyond the limit no arrest occurred although slowing of the rhythm might be observed.

The form of the Arrest.—This was generally in full inspiration, or at some point midway between inspiration and expiration. At the centre of the area arrest occurred sometimes in active expiration or in expiration.

The arrest, especially when the stimulus was sufficiently strong and applied near the centre of the area, could be maintained for 6-10 seconds, and after the cessation of the stimulus respiration commenced with a slow rhythm and only gradually regained its former rate. The experiment could be repeated again and again either at the same place, or at various points of the area.

Tracing I.
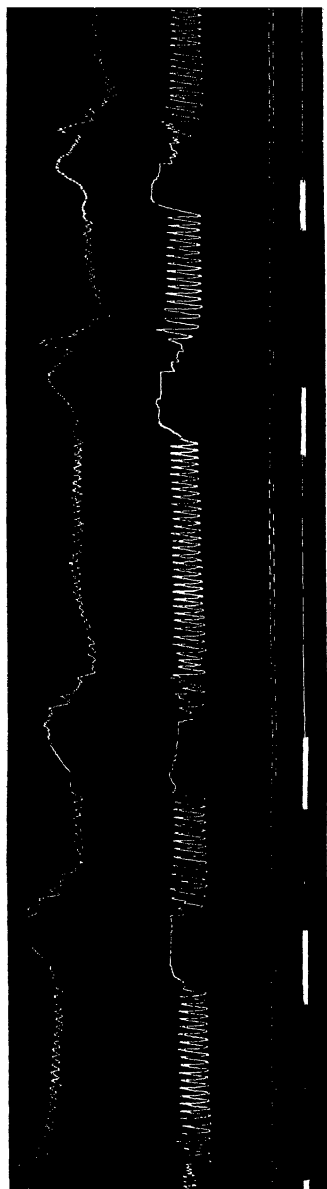
Description of Tracings showing Slowing and Arrest in the Cat.

Tracing 1, p. 621.   The point of the cortex excited was the central point of the area.   See Plate 57, Photograph II.

The first line is the manometer tracing, the second that of the respiration, the third marks the rate of the travelling surface, the space between each vertical stroke being traversed in a second, the fourth the incidence, duration, and amount of the stimulus.   With 6 the respiration was slowed or incompletely arrested, with 5 arrest took place in full inspiration, and this was repeated four times at short intervals.

Arrest in expiration could not be obtained so commonly in the cat as in the dog. The arrest in active expiration appeared to be limited to the centre of the area and was evoked by a current which did not influence any part beyond the exact spot mentioned.   Moreover for this result it is essential that the effects of shock should be avoided as far as possible.

I will now give a table formed from tracings taken from most of the points of the area within which arrest of respiration can be obtained.   All the tracings were taken from the same cat.   Other excitation experiments made on the cortex of the same animal beyond the "arrest" area were negative.   The arrest took place in full inspiration throughout, near the olfactory bulb the arrest was broken by the over-inspiratory clonus to be described later on.   I also add a diagram made by enlarging Photograph II on which the numbers indicate the points stimulated.

Diagram I.
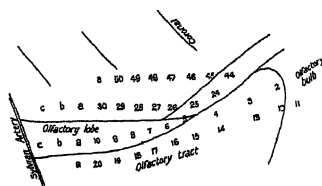
It is not maintained that there is a sharply defined limit beyond which a strong stimulus has no effect, but that the area described is the most extensive met with in favourable cases, the more deeply anæsthetized or the more exhausted the animal tho more does the area contract.

TABLE.—See Diagram I. for points.

Amount of Faradic current.	Points resulting in	
	Arrest.	Slowing.
8	8 incomplete 9 ,, 26 27	7 10 (a) 10 (b) 25
7	6 7 8 9	
6	2 3 10 10 (a) 10 (b) 11 25 28 29 30 30 (a) 49	16 30 (b) 47 48
4	4 5 10 (a) 12 15 16 17 18 30 (b) 48	14 19 46
2	14 46	10 (c) 13 30 (c)
0	10 (c) incomplete 13 24 incomplete 30 (c) incomplete 45 50	20 44 50 (a)

Points.	Amount of current	Result.
2	6	Arrest, " clonus."
3	6	" Clonus," then arrest
4	4	" Clonus," then arrest
5	4	Arrest.
6	7	Arrest.
7	8	Slowing.
7	7	Arrest.
8	8	Arrest, incomplete.
8	7	Arrest.
9	8	Arrest, incomplete.
9	7	Arrest.
10	7	Slowing.
10	6	Arrest.
10 (a)	8	Slowing.
10 (a)	6	Arrest, incomplete.
10 (a)	4	Arrest.
10 (b)	8	Slowing.
10 (b)	6	Arrest.
10 (c)	2	Slowing.
10 (c)	0	Arrest, incomplete.
11	6	Arrest.
11	4	" Clonus."
12	4	Arrest.
13	2	Slowing.
13	0	Arrest.
14	4	Slowing.
14	2	Arrest.
15	4	Arrest.
16	0	Slowing.
16	4	Arrest.
17	4	Arrest.
18	4	Arrest.
19	4	Slowing.
20	0	Slowing.
24	0	Arrest, incomplete
25	8	Slowing.
25	6	Arrest.
26	8	Arrest.
27	8	Arrest.
28	6	Arrest.
29	6	Arrest.
30	6	Arrest.
30 (a)	6	Arrest.
30 (b)	6	Slowing.
30 (b)	4	Arrest.
30 (c)	2	Slowing.
30 (c)	0	Arrest, incomplete.
44	0	Slowing.
45	0	Arrest.
46	4	Slowing.
46	2	Arrest.
47	6	Slowing.
47	4	Arrest.
48	6	Slowing.
48	4	Arrest.
49	6	Arrest.
50	0	Arrest.
50 (a)	0	Slowing.

Slowing and Arrest of Respiration by Excitation of the Cortex Cerebri of the Dog.
    See Photograph III.

The Necessary Stage of Anæsthesia.   See p. 619.

When the animal was strong a little chloroform was mixed with the first ether used, but not afterwards.   In any case ether has to be given in a more concentrated form to dogs than to other animals.   The employment of the small doses of morphine, mentioned on p. 620, is not absolutely necessary, and I did not use it until I had found the area, but it is certainly convenient for the prevention of sudden variations in the anæsthesia.

The Strength of the Stimulus.—At the centre of the area arrest was generally best obtained in the dog with 6, but in some instances 7 proved sufficient, others required 5.   Along the outer limit 0 was necessary, and there were intermediate stages where arrest took place with 4 and 2.   Just outside the border slowing could be produced with 0.

The Form of Arrest.—This was generally in active expiration, particularly at the centre of the area.   Less often the arrest was in expiration.   In the stage of anæsthesia employed it was only sometimes that arrest in any degree of inspiration occurred, but the tendency was more marked the further the distance from the centre of the area.

Tracing II., pp. 626 and 627.   For the three points excited see Photograph III*a*, *b*, *c*.

Arrest in active expiration was produced from three central points of the area excited in succession.   In each case 8 produced slight slowing, but 6 was required to arrest.   Some irregularity of rhythm with an increase in amplitude took place after the cessation of the excitation before the respiration resumed its regular character.

Tracing III., p. 630.   For the point stimulated see Photograph III.

The experiment in this case was made at the anterior border of the area and arrest was only produced at 0.   The arrest did not last the whole time of the stimulus, but during the latter part the breathing started again slowly.

Slowing and Arrest of Respiration by Excitation of the Cortex Cerebri of the Rabbit.
    See Photograph I*a*.

The Necessary Stage of Anæsthesia.   See p. 619.

Ether alone was, as a rule, used in this animal in sufficient amount to prevent voluntary and reflex movements from occurring when the spot was excited, but no more.   Even minute doses of morphine reduced the excitability enough to prevent any arrest of respiration from being obtained.

The Amount of the Stimulus.—At only one spot could arrest of respiration be obtained, and that with 8 or 6.   Stronger currents applied around this spot did not cause arrest.
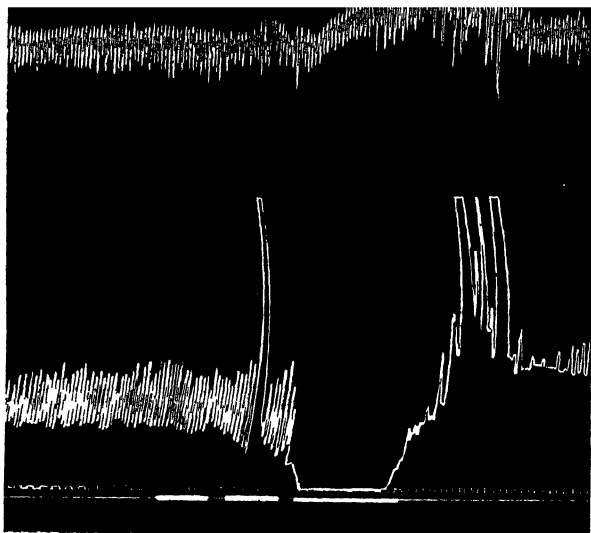
10   8   6

Tracing II. (a) 2.

Tracing II. (b).

10   · 8   6
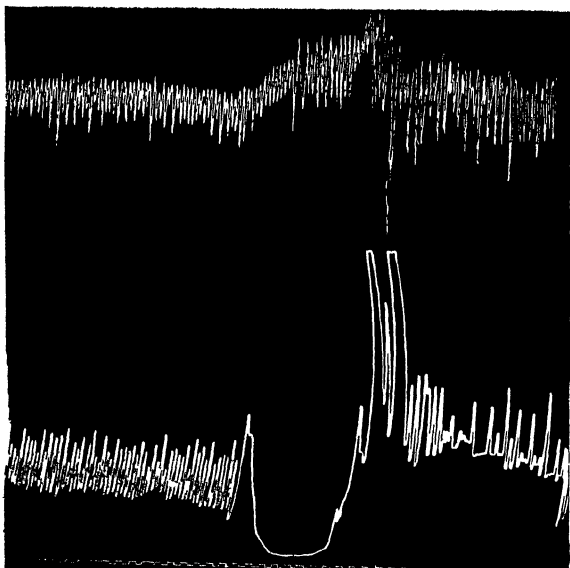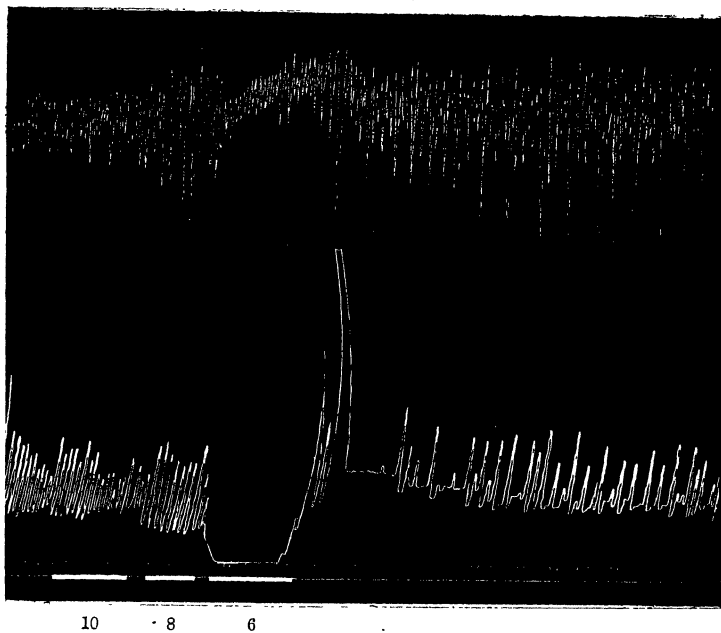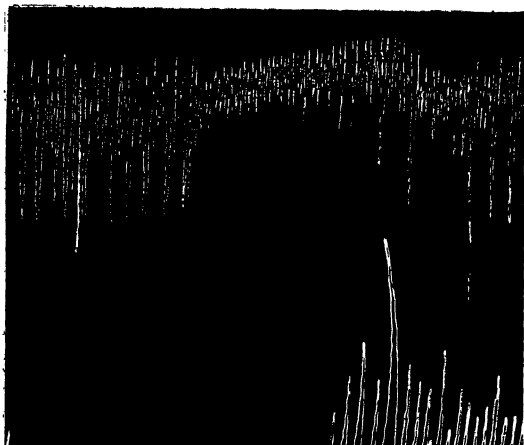
Tracing II. (c).

The Form of the Arrest.—This was nearly always in full inspiration, but as in the cat arrest in expiration occasionally happened.

Tracing IV., p. 630.   The point excited is marked on Photograph I*a*.

The respiratory rate was slowed with 10, and arrested in full inspiration at 8.

Slowing and Arrest of Respiration by Excitation of the Cortex Cerebri in the Monkey (Macacus Rhesus).

The Suitable State of Anæsthesia.   See p. 619.

The Exposure of the Orbital Surface of the Frontal Lobe.—The animal being placed on its back in a semi-recumbent position with the head fully extended, the orbital surface of the frontal lobe appeared facing forwards and upwards when the frontal bone including the orbital plate was removed.

The Blood-pressure Tracing.—The carotids were not interfered with in order to avoid complications from the effects illustrated by the experiments contained in a former paper.*   Therefore the cannula in the monkey was invariably placed in the common iliac artery outside the peritoneum.

The Arrest Area.—This has been marked out in Photograph IV.   I do not assert that the effect cannot be obtained at points internal to the chord of the arc drawn. But there are two difficulties in trying to decide this point, firstly, the liability of wounding the internal carotid artery and its branches, and secondly, the impossibility of preventing the spread of the excitation to the meninges.   I prefer, therefore, not to absolutely delimit the inner border of this region.

The Degree of the Stimulus.—A current of 7 or 6 arrests respiration when applied just external to the junction of the olfactory tract with the uncinate convolution. But exciting points 1 millim. apart along any radius of the semicircle indicated in the photograph, arrest of respiration occurred with 4, 2, or 0, according to the distance from the centre.   Just beyond the border 0 caused slowing, but not arrest.

The Character of the Arrest.—The arrest in the monkey was nearly always in expiration, but only rarely was any active expiration seen.   Beyond the disappearance of the respiratory curves no change was noted in the circulation from excitation of the cortex only.

Tracing V., p. 631.   The points excited are in connection with Photograph IV.

(*a*) Respiration which was not arrested by 8 was stopped by 7.   This was at the centre of the area.

(*b*) 0 was required in this experiment, the point excited being at the anterior border of the area.

(*c*) A little within the limit 2 was necessary.

(*d*) Just outside the border there was no arrest with 0.

* SPENCER and HORSLEY, "The Control of Hæmorrhage from the Middle Cerebral Artery," British Medical Journal, 1891, vol. I., p. 457.

It is worthy of note that Tracing V., *a, b, c, d* were from the same monkey, some other similar experiments intervening.

The Occurrence of the Arrest.

It has, therefore, been shown that the respiration can be slowed and arrested by excitation of a certain spot and a limited area around it. This spot is situated in all the animals examined to the outer side of the olfactory tract just in front of the junction of the tract with the uncinate. And this arrest can be constantly obtained and the experiment repeated again and again under certain conditions.

1. The animal must be in such a stage of anæsthesia, that whilst the other effects upon respiration hereafter to be described are excluded, the excitability of the cortex for the arrest effect shall not be lost.

2. The animal must be in a normally active stage, and the brain to be excited must not have been injured in any way.

3. A sufficiently strong faradic stimulus is required.

Conversely the Causes which prevent the Occurrence of the Arrest.

1. (*a*) *Insufficient Anæsthesia.*—Over-inspiration and over-inspiratory tonus with slowing or acceleration of rhythm are excited when the narcosis is not of the right degree and arrest is prevented. In many cases the tracing thus obtained strongly suggested the struggle between the conflicting effects, *e.g.*, excitation sometimes produced a short arrest, which then became arrest in over-inspiration—and near the olfactory tract a clonic effect occurred instead of arrest. If the animal were allowed to awake sufficiently there might ensue reflex arrest for a second or two on exciting with a weak current, but a repetition of the excitation under these circumstances caused a different response.

1. (*b.*) *Too Deep Anæsthesia.*—With ether sufficient to abolish the corneal reflex, the cortex of this region was rendered inexcitable. At the centre of the area 0 might produce slowing but no arrest, even this was lost in deeper anæsthesia. Morphine, when given in larger doses than those mentioned, likewise abolished the excitability, the respiratory rhythm tended to become irregular, showing the phenomena of periodic respiration, of spontaneous pauses in expiration, and of convulsions. This was especially the case in the dog, but was also observed in the rabbit and monkey. When the animal was subjected to sufficient morphine for spontaneous arrests to occur, arrest of respiration, as others have noted, could easily be obtained by faradizing any sensory surface, including the convex surface of the brain, but was equally well got from a nerve such as the sciatic.

2. The cortex of animals not in a normal state of health before the experiment was more easily inhibited by the ether, and also the cortex was less excitable, *e.g.*, slowing, but no arrest, might be obtained. In other words, the depression due to imperfect health was added to the ether effect, and in some cases was sufficient

Tracing III.
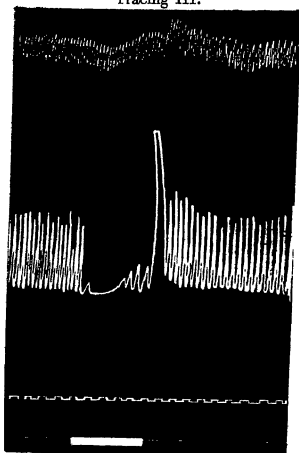
0

Tracing VI.
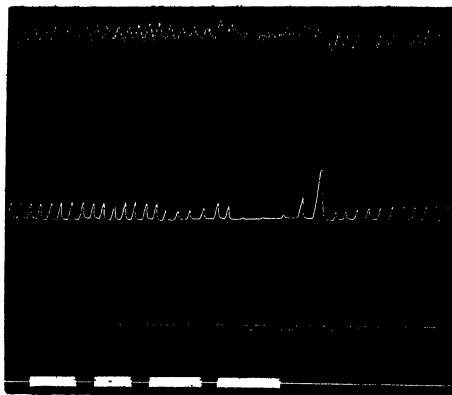
8   7   6   5

Tracing IV
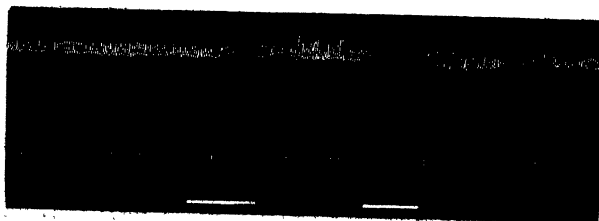
10   8

Tracing VII.

Tracing V. (a).

8   7

Tracing V. (b).

0

Tracing V. (c).

2

Tracing V. (d).

0
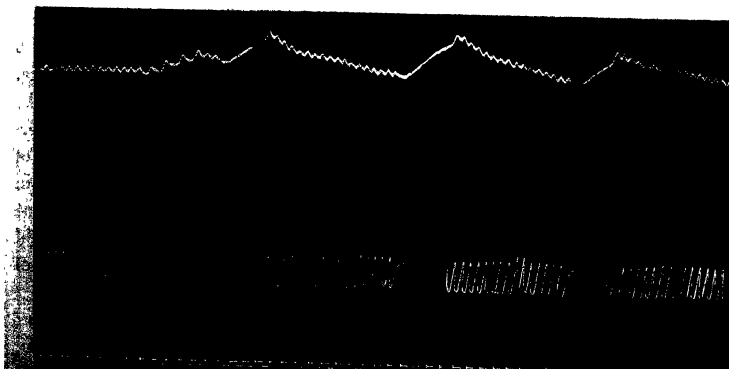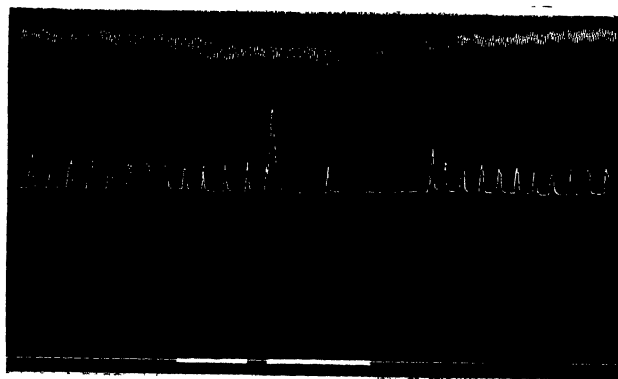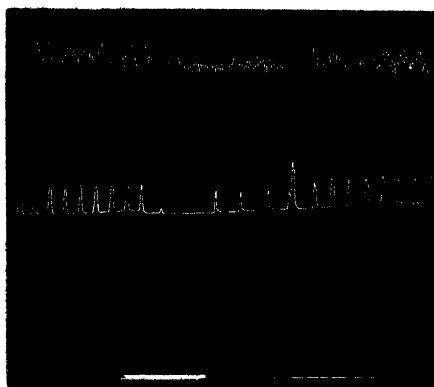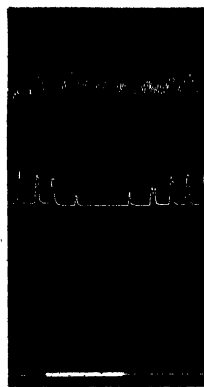
of itself to abolish the excitability. Such instances occurred in monkeys with diarrhœa, in dogs with tape-worms, in rabbits with parasites in the liver, and in pregnant cats. The latter condition was not sufficiently advanced in any case to be noted before the experiment, and there was, of course, no mechanical obstruction to the respiratory movements. Some of the pregnancies noted were very early.

Exhaustion of the animal from exposure of the brain, from loss of blood, from repetition of the experiment, tended to diminish the excitability, and this ensued all the more rapidly the deeper the primary anæsthesia, whether from ether or from the prior causes above noted.

3. No arrest could be obtained except when the secondary coil was at least 8 or 7 centims. from the primary, although slowing of the rhythm might occur with a slightly weaker stimulus.

(b.) On the connection with the Medulla Oblongata of the area of the Cortex Cerebri for Slowing and Arrest of Respiration.

I will now proceed to describe the results of the excitation method I have employed to trace the fibres connecting the cortex with the central mechanism in the medulla oblongata.

On this point I need not describe in full detail the results obtained in each of the species of animals examined. Since the tract, connecting the area on the cortex with the medulla, runs through the ventral portion of the brain, i.e., through a part in which the difference between the four species is very slightly marked, and also because the results were practically identical. But although I have combined the description and inserted in this paper only a few tracings, yet I have, in my experiments, assumed nothing. I have stimulated every hemisection in each species of animal many times, and have tracings sufficient to afford many illustrations of every point.

Method of making Hemisections or Sections of the Brain. See p. 613.

The necessary state of Anæsthesia. See p. 619.

The deepest ether anæsthesia consistent with regular respiration was found to be the best. Of course great watchfulness was required to prevent an overdose. In some cases, when once arrested, the respiration did not begin until after some artificial respiration had been employed, but usually the respiration started again spontaneously. Only in some rabbits did the respiration tend to stop before they were sufficiently anæsthetized. I used, in these exceptional cases, the small dose of morphine already noted, viz., 0·01 grain per 1 lb. live weight, and so obtained the necessary anæsthesia without an excess of ether. As already mentioned, the dog required the ether vapour in a more concentrated form than other animals.

Tracing VIII. (*a*)
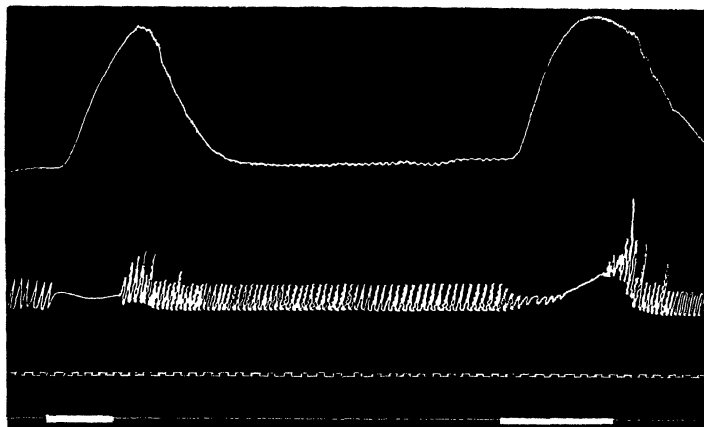
Tracing VIII. (*b*)
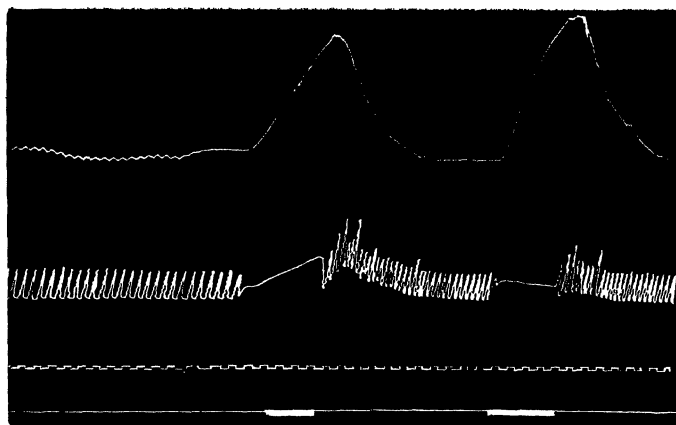
Mode of Experiment.—A vertical transverse (frontal) hemisection (sometimes a section) having been made in a deeply narcotized animal, and the point to be excited located and dried, the electrodes were held in position whilst a tracing of the circulation and respiration were being taken. The current was then applied for 6 seconds or more ; generally current 6, sometimes 7 or 5, was required to produce arrest. The record was continued until the recovery of the rhythm to the state existing before the experiment. When the electrodes were placed on the right spot and arrest obtained, the electrodes being held *in situ*, the experiment could be repeated again and again for six times or more with the same result.

The Form of the Arrest.—Arrest generally took place in full inspiration, less often in a position midway between inspiration and expiration. It was only in the deepest stages of anæsthesia that arrest took place in expiration, especially in monkeys. It was difficult to keep the other animals in this sufficiently deep stage whilst breathing regularly, or, if arrested by excitation, respiration required artificial aid to start again.

The Strand of Fibres which, when excited, produced Arrest.—If the series of photographs taken from Dr. Tooth's sections of the brain of the cat be examined, and the fibres enclosed in the ring noted (see Plates 58 and 59), it will then be possible to trace the following description :—

From the centre of the cortical " arrest" area, in each animal fibres may be followed back, and these are observed to be gathered together at the lower and anterior end of the lenticular nucleus, and apparently form part of the bundle known in human anatomy as the olfactory limb of the anterior commissure. This bundle passes backwards, upwards, and inwards, to the inner side of the anterior cornu of the lateral ventricle. These fibres tend to the middle line, at which point they form the more anterior fibres of the anterior commissure. Evidence that these fibres actually decussate with those of the opposite side will be referred to directly. Behind the anterior commissure, the tract passes downwards and outwards from the middle line, close to the infundibulum, above the optic commissure, and then above the inner end of the optic tract. Behind this, it runs above and just internal to the crusta. At the beginning of the aquéduct, the strand runs backwards in the tegmentum, just external to the fibres of the 3rd nerve as they pass towards the ventral surface. At the level of exit of the 3rd nerve the fibres lie vertically above the point of exit of the nerve, being an equal distance below and outside of the aqueduct in the structure known as the red nucleus. The strands on either side are here parallel ; as far as the exit of the 3rd nerve the bundle of fibres can be traced microscopically, but, beyond, they become lost amongst the other fibres of the tegmentum. But evidence can be obtained experimentally of their parallel course as far back as the upper border of the pons. Beyond this I have not traced them. The animals used in the present research were adults, and in them, when a hemisection was made involving the pons fibres, the respiration either became irregular or very slow. It is possible that younger individuals would be more tolerant of disturbance. Two variations in the method

of experiment went to show that the strand on each side really decussates at the anterior commissure. Thus (1) if the brain be hemisected frontally and removed as far back on one side as the origin of the 3rd nerve, the other half of the brain is thus exposed on its mesial aspect, so that either the cortical mesial surface or the cut surface can be excited. There is one point, and one point only, on the mesial aspect where arrest can be produced by excitation, and that is the cut surface of the anterior commissure, and immediately behind and below this structure. The fibres which have come from the cortex of the side removed, are in this way excited immediately after decussation. In the monkey the decussation of the so-called olfactory limb of the anterior commissure can be easily perceived to take place in front, and can be clearly distinguished from the rest of the anterior commissure which joins the temporo-sphenoidal lobes. Again, if after hemisection and extirpation at the level of the 3rd nerve the cortex of the opposite side be excited, no arrest can be obtained, nor can any arrest be obtained from hemisections made in this remaining half in front of the anterior commissure. By using very strong currents some slight slowing may occasionally be observed.

Description of Tracings to Show the Result of Excitation of Cerebral Hemisections in Producing Slowing and Arrest of Respiration.

Tracing VI., p. 630. Monkey. From a hemisection immediately behind the cortical arrest area. See Photograph V. Coil 6 caused slowing with diminished amplitude; 5, arrest in expiration.

In the dog, arrest in active expiration and in expiration was obtained with a tendency towards an inspiratory phase when a stronger stimulus was used.

Tracing VII., p. 630. Cat. From a hemisection at level of Photograph VII. where the "olfactory limb of the anterior commissure" first appears as a distinct bundle. Arrest occurred in inspiration.

Tracing VIII., p. 633. Rabbit. From a transverse section through the anterior commissure, *i.e.*, the electrodes were applied to the anterior commissure in the middle line. See Photograph IX. The respiration was arrested four times in succession with 5. There was each time a great rise of blood pressure. It is to be noted that the rabbit could not, without risk, be always so deeply anæsthetized as the other animals. On the contrary, in the monkey this is easily accomplished, and then it will be seen that no rise of blood pressure occurred. See Tracing IX.

Tracing IX., p. 636. Monkey. From the mesial surface of the left hemisphere after the removal of the right half behind the level of exit of the 3rd nerve. The electrodes were applied just behind and below the cut surface of the anterior commissure. See Photographs IX. and X. Arrest was obtained in expiration twice with 6 and six times in succession with 5.
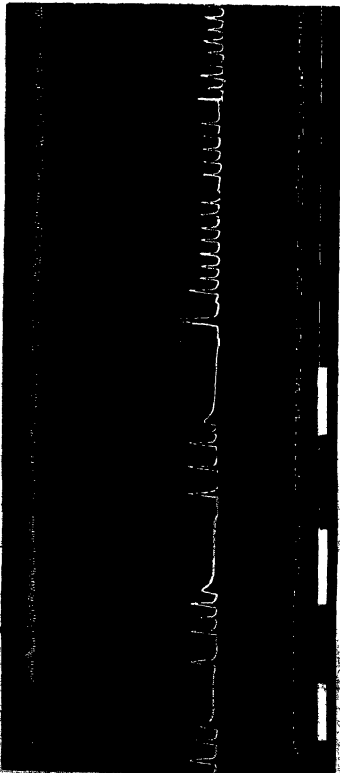
Tracing IX. (b).
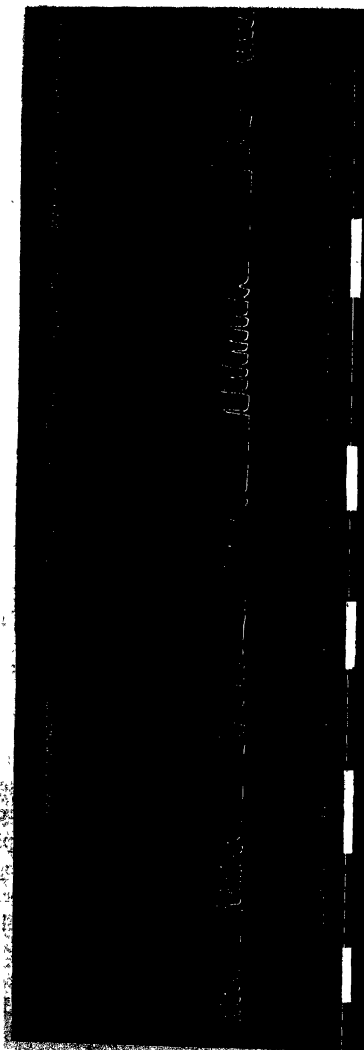
Tracing X. (a).
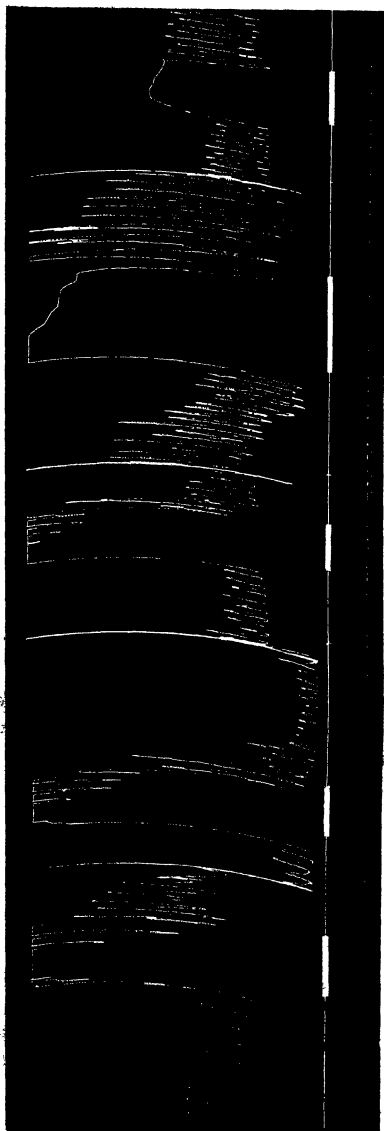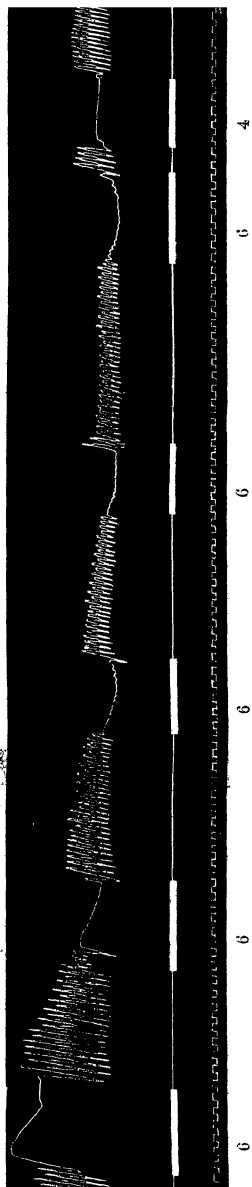
Tracing X. (b).

Tracings VIII., IX., and XI. illustrate an important point, viz., that the results described can be obtained many times in succession by keeping the electrodes to the same spot, and making use of a stimulus sufficiently strong, yet not so great as to quickly induce exhaustion, provided always that the animal be kept in the same state of anæsthesia.

In the case of the experiments illustrated by the next tracing, a variation in procedure was adopted, in order to show the influence of the etherisation. The animal was first allowed to come out a little from the state of deep anæsthesia, and then excitation was repeated whilst ether vapour was being administered, so that the animal was in a more deeply anæsthetized state with each experiment than it had been in the previous one.

Tracing X., p. 637. From a hemisection in a dog at the level where the optic tract is cut longitudinally in its course to the occipital cortex. See Photograph XI. The animal during the time that the record was being taken was breathing ether and becoming more deeply anæsthetized. Arrest was produced eleven times in succession at the same spot in an animal which was at the commencement in a superficial stage of anæsthesia, but, by breathing ether, gradually passed into a deep stage. In the first experiment the arrest was complicated by the over-inspiratory tonus, which will be referred to later on. In the second experiment the over-inspiratory tonus tending to complicate the arrest was again present, but weaker. The tracing also illustrates a commonly observed point, that with the deepening of the anæsthesia some increase in the strength of the stimulus was required. Thus the first experiment required 3 and the last 4, but allowance must be also made for exhaustion due to the repetition of the experiment at short intervals.

Tracing XI., p. 639. From a monkey at the level of exit of the third nerve, i.e., by exciting the centre of the red nucleus. Arrest was obtained at the point indicated six times in full inspiration. See Photograph XIV.

Tracing XII., p. 642. Rabbit. From a hemisection behind the level of exit of the third nerve and immediately above the pons. See Photograph XV. Arrest was obtained three times with 6 and four times with 5, each time in full inspiration.

Tracing XIII., p. 642. Dog. From the mesial surface of the left hemisphere behind and ventral to the anterior commissure after right hemisection and removal behind the level of exit of the third nerve. The arrest in quarter to half inspiration was maintained by 0 for 50 seconds. The tracing shows that the arrest of respiration could be maintained for long periods by using strong currents. Of course, exhaustion was produced in this manner.

Remarks on the above Results from the animal.—Unless the anæs- . . . over-action such . . . and over-inspiratory prevents the . . . on masks . . . The electrodes must be . . . and any . . . to the long axis of the . . . small deviation is sufficient to cause failure. When the

Tracing XI. (a).
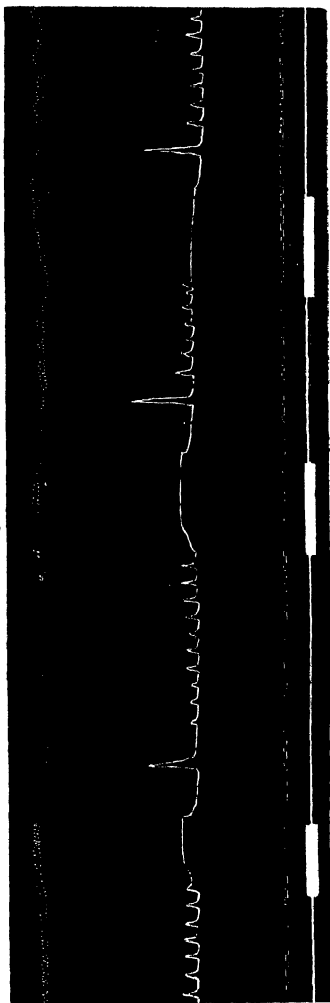
Tracing XI. (b).
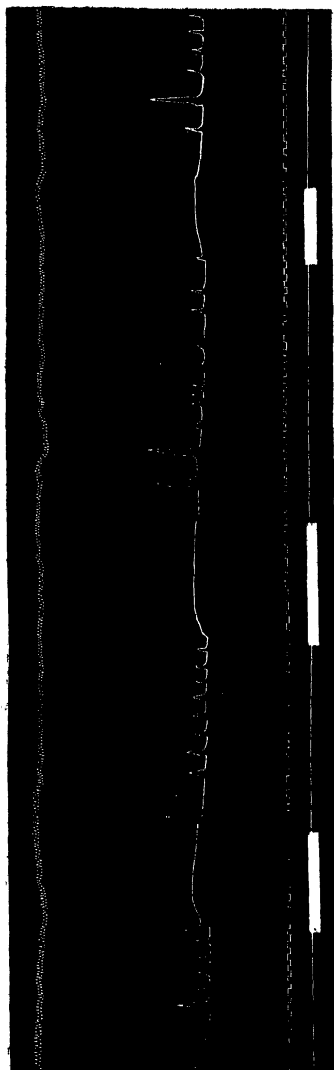
electrodes are applied exactly to the right place and held steadily there, the experiment may be repeated many times in succession. Exhaustion with slow or irregular rhythm does not abolish the excitability, but the arrest being produced, it tends to continue even after the cessation of the stimulus, and artificial respiration may be required to start the rhythm.

THE DETAILS OF THE EFFECTS OBTAINED BY EXCITATION OF THE CORTEX CEREBRI, AND OF THE SURFACE OF VERTICAL SECTIONS THROUGH THE CEREBRUM, IN THE CAT, DOG, AND RABBIT.

B. An Increased Action.

(1.) *Acceleration of the Rhythm.*

General.—The excitation of any sensitive part in a non-anæsthetized animal tends to disturb the respiration in the direction of increased action, the rhythm is more or less accelerated, irregular, and there are muscular movements of the limbs and trunk. In a slightly anæsthetized animal a strong stimulus produces this effect over a wide extent of the cerebral surface. Under ether anæsthesia sufficient to abolish the excitability of the cortex so far that no movements of the limbs are evoked by the stimulus, acceleration of respiration is not caused by faradic excitation except by excitation of one spot on the cortex. Even if the faradic current be so strong as to call forth automatic movements, any acceleration of the rhythm which then may be obtained elsewhere is much less pronounced than the rapid rhythm provoked by exciting this particular spot.

On stimulating hemisections of the brain from before backwards this acceleration area on the cortex can be found to be connected with the medulla along a definite line, excitation of any point along this line producing marked acceleration.

So long, therefore, as the animal is sufficiently under the anæsthetic, acceleration of rhythm only occurs on the stimulus being applied at the one spot on the cortex and along the course of the tract through the cerebrum.

The Suitable Stage of Anæsthesia.—In all three species of animals on which the following observations were made, sufficient ether was given to abolish the excitation of movements of the limbs, and to suppress the over-inspiratory tendency which will be later on referred to. This applies both to the excitation of the cortex and to that of the surface of the sections of the hemisphere. A deep stage of anæsthesia abolished all cortical excitability for acceleration, as well as that of the sections.

One source of error had always to be carefully excluded, viz., the alteration in the activity centre due to apnœa produced by the acceleration. It was therefore found to only allow the stimulus to act for about six seconds, otherwise the rhythm began to get slower. It was also necessary to allow full time for normal respiratory rate before repeating the experiment, otherwise it was found to have been lowered by the apnœa

The Area on the Cortex Cerebri where the most marked Acceleration was obtained on Excitation.

In the Dog and Cat.—The effect was best marked at the upper end of the supra-orbital sulcus. From a limited area around the acceleration could be obtained, but in a less marked degree. On increasing the anæsthesia this was the last place where any acceleration could be obtained with 0, and it was at this point that acceleration could be first obtained in an animal recovering from deep anæsthesia.

In the Rabbit.—If, when viewing the dorsal aspect of the rabbit's brain, the eye travels back from the olfactory bulb along the inner margin of the hemisphere, an artery is seen coming up between the edge of the hemisphere and the falx cerebri, and then turning over on the convex surface and running outwards in a line which suggests the position of the crucial sulcus in the cat and dog. When the pia mater is carefully stripped off, a groove remains. This vessel serves to indicate the place where marked acceleration can be obtained in this animal. When the electrodes are placed astride of the vessel near the margin marked acceleration is obtained. An area of 2 millims. in diameter overlapping the margin, *i.e.*, extending a little way on to the mesial surface, is about the limit within which this phenomenon is markedly represented.

The Connection with the Medulla Oblongata of the Area on the Cortex which causes Acceleration when Excited.

The course which the fibres leading from the cortex cerebri take towards the medulla oblongata is indicated upon the accompanying photographs by a cross. It descends in the corona radiata to the lenticular nucleus where this structure blends with the lower and external part of the internal capsule as seen in frontal sections. The band of fibres then passes below the internal capsule as the latter changes into the crusta, and appears to slope towards the inner portion of the tegmentum. The strand on each side reaches the middle line in the same frontal plane as the exit of the 3rd nerve. The spot where acceleration is at this level obtained is thus in the inter-peduncular grey matter, rather nearer to the ventral margin than to the floor of the aqueduct.

The acceleration tract on either side appears to decussate in the plane of the exit of the 3rd nerve. I have already shown that hemisection and extirpation on one side behind the anterior commissure, but in front of the plane of exit of the 3rd nerve, removes the "arrest" effect; but marked acceleration can still be obtained from the spot on the cortex of the remaining hemisphere. On the other hand, removal of one hemisphere immediately in front of the pons does away with the special acceleration from the cortex of the remaining hemisphere. Some slight acceleration may still be obtained from the cortex and internal capsule if the anæs-

Tracing X

Tracing XIII.

Tracing XIV.

8   7

Tracing XVI.

8   6

Tracing XV.

6   6   6

4 N 2
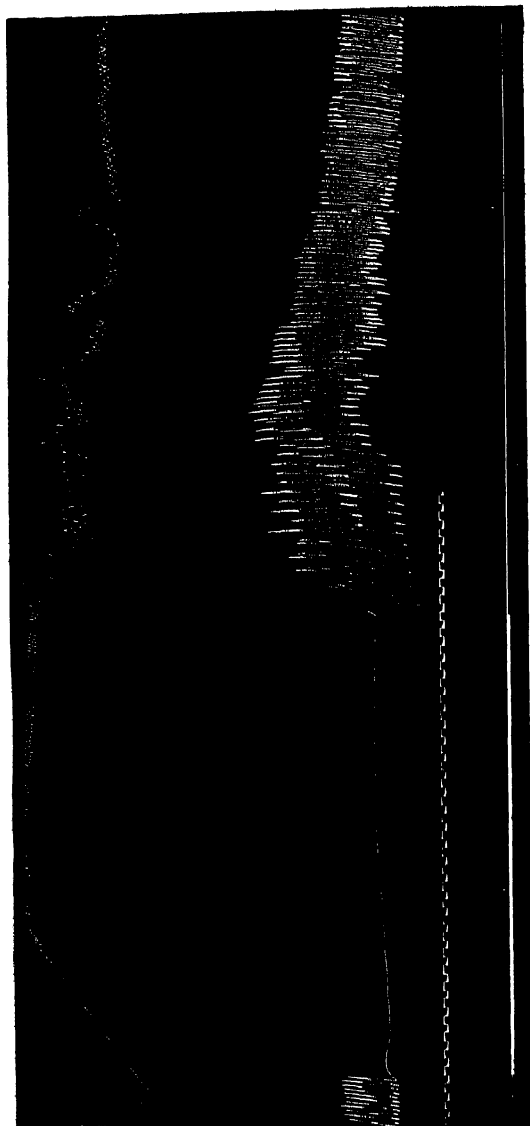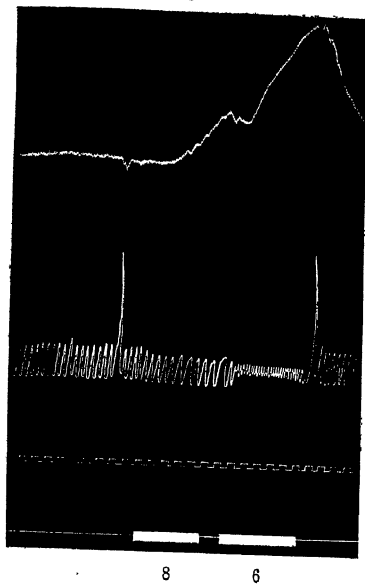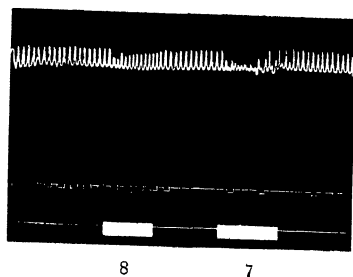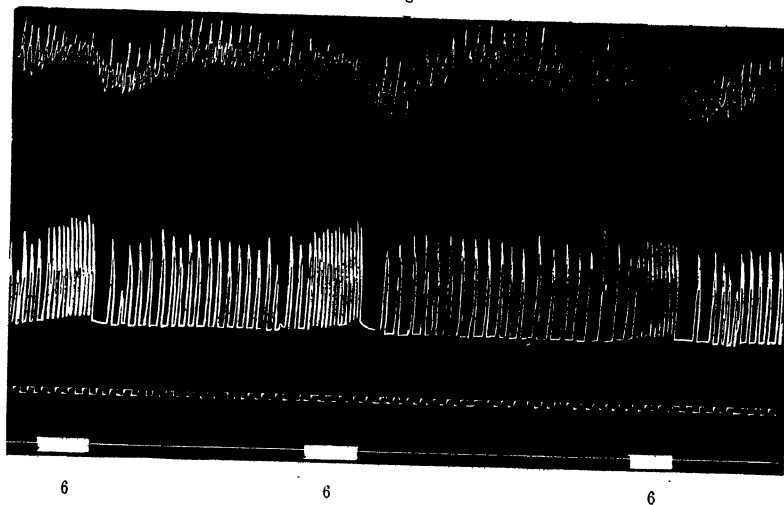
thesia become superficial, but this is the general effect mentioned on page 640, which is only very small, and is connected with the medulla through the descending sensori-motor tract, *i.e.*, does not decussate above the pons. Finally, when one-half of the cerebrum has been removed no marked acceleration can be obtained from the mesial surface of the remaining half, except from a point immediately dorsal to and behind the exit of the 3rd nerve from the crus.

The Description of Tracings showing Acceleration.

The respiratory rate was counted by comparing a period of 6 seconds before and during the stimulus.

Tracing XIV., p. 643. Rabbit. The spot on the cortex cerebri of the right side was excited (see Photograph IB). Before any excitation the respiratory rate was 80 per minute. Stimulation with 8 caused the rate to become 100 per minute, *i.e.*, an increase of 0·25. With 7 the rate became 120 per minute, *i.e.*, an increase of 0·5.

Tracing XV., p. 643. Dog. Excitation of the upper end of the supraorbital fissure on the left side, after hemisection and extirpation of the right half behind the level of the anterior commissure. Excitation with 6 caused acceleration three times in succession, the first time from 50 to 110, an increase of 1·2, the second time from 40 to 120, an increase of 2, and the third time from 35 to 95, an increase of 1·7. The slow rate of respiration before the stimulus marks a deeper stage of anæsthesia than Tracing XXI. Further, also, an opposite hemisection having been made in this case, it is clear that the fibres connecting the cortex with the medulla cannot cross above the level of hemisection.

Identical effects were obtained from the cat.

Tracing XVI., p. 643. Cat. For point of excitation see Photograph VIII. With 8 no acceleration occurred, but with 6 the rate changed from 70 to 170, an increase of 1·4. There was a marked rise of blood-pressure; in other experiments with deeper anæsthesia no rise in blood-pressure occurred (see Tracing XVIII.), and the respiration being slower at the commencement, even greater acceleration followed. I have a tracing from a dog, taken from the same spot. 8 produced a fractional increase of 3·4, viz., from 25 to 110 ; the absolutely greater rate occurs in the slighter stages of anæsthesia, *e.g.*, 170 in this Tracing.

Tracing XVII., p. 645. Rabbit. Right hemisection through the plane of the exit of the 3rd nerve, see Photograph XIV. With 8 the increase was from 60 to 120, *i.e.*, an increase of 1 on the first experiment and an increase from 45 to 150 on the second experiment, *i.e.*, an increase of 2·3. The second experiment was thus better than the first. It has often been found that an almost imperceptible movement of the electrodes may, on the one hand, improve the reaction and, on the other hand, impair it. This indicates the necessity of exciting the exact point if the maximal effect is to be obtained.

Tracing XVII.

Tracing XVIII., p. 646.   Dog.   Right hemisection at the plane of exit of the 3rd nerve.   See Photograph XIV.   The rate increased from 55 to 110, *i.e.*, 1, with 10. There was practically no variation in blood-pressure.   In this region where the electrodes were so close to the crusta, the animal had to be well anæsthetized, although not so deeply as required for the arrest of respiration, for if not, over-inspiratory tonus complicated the acceleration, and there was at the same time a marked rise in blood-pressure.

(2.) *Over-inspiratory Clonus.*   (*" Snuffing" Movements.*)

This movement produced by excitation was similar in the several species of animals and was of the following character.   The animal made an over-inspiration, and then several sharp over-inspirations were superimposed upon the primary one. The over-inspiratory jerks were peculiar in following one another at regular intervals in a rhythmic manner and in not ceasing exactly at the same time with the stimulus, one, two, or three more of these over-inspirations taking place after the cessation of the stimulus.

The result could be obtained from a definite area of the cortex, and along a tract down to the medulla.

The Area on the Cortex for Over-inspiratory Clonus.

This was invariably and most easily obtained from the junction of the olfactory bulb and tract.   By excitation of the portion of the frontal lobe, "prorean lobe," lying immediately above and behind this spot the same effect could be obtained by means of a stronger stimulus.   But the typical effect with the weakest excitation was obtained at the junction referred to, both on the dorsal and lateral aspect.

The Apparent Course of the Fibres Connecting the Cortex with the Medulla by the Excitation of which Over-inspiratory Clonus is produced.

This was followed in the vertical sections commencing with those of the outer limb of the olfactory tract, and continuing backwards past the furrow formed by the

Tracing XVIII.

Tracing XX.

10
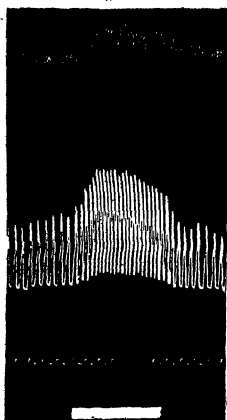
8
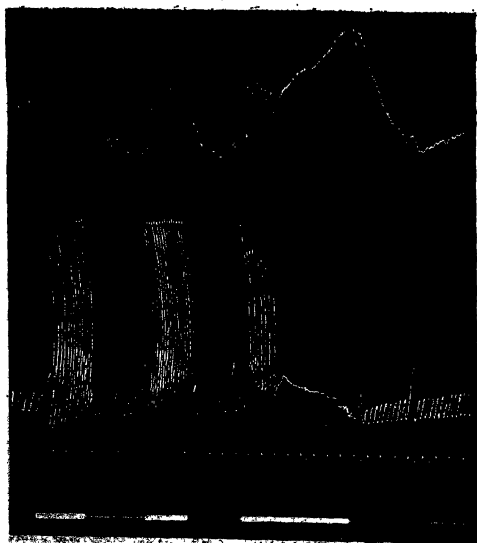
Tracing XIX.

6   4   2

Tracing XXI.
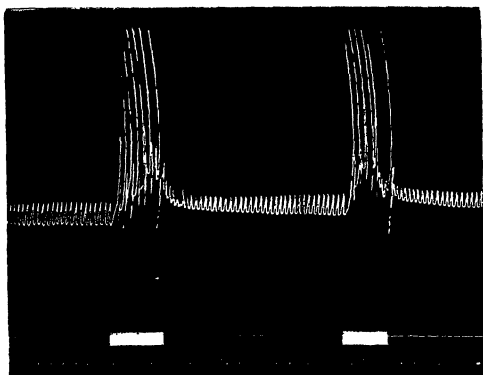
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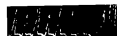
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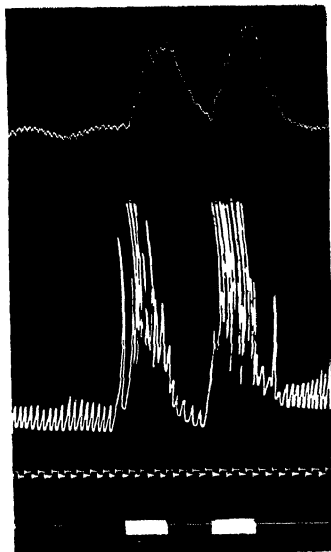
sylvian artery to the temporo-sphenoidal lobe at its mesial part, viz., the uncinate convolution. The effect obtained by excitation was traced along the uncinate convolution to the uncus. In the vertical section the over-inspiratory clonus is thus obtained from the region outside the optic tract as it passes backwards from the chiasma, whereas all the other respiratory effects are represented in this section internal to the tract. In the next section, behind where the optic tract has extended to the occipital lobe, the over-inspiratory clonus is to be obtained from the outer side of the peduncle, external to the crusta. Immediately behind the exit of the 3rd nerve transverse pontine fibres begin to separate the crusta from the ventral surface, and over-inspiratory clonus can here be obtained beneath the pyramidal tract. Behind the posterior perforated spot, at the naked-eye margin of the pons, the clonus can be obtained in the middle line at the ventral margin.

The course of the fibres which seem to subserve this reaction was easy to trace as far backwards as the section through the optic tract, see Photograph XII. Naturally, however, the connection between the uncinate gyrus and the region of the crusta, &c., was involved in greater difficulty. I repeated the experiments on this point whilst constantly referring, as I have always done throughout this research, to Dr. Tooth's microscopical preparations of the cat's brain and to the photographs made from them. In the section from which Photograph XIV. was taken transverse or oblique fibres are already apparent beneath the crusta, although the 3rd nerve is not yet passed, and the posterior perforated spot still forms the ventral margin in the middle line.

The excitation of the under-surface of the anterior border of the pons was the one spot where the over-inspiratory clonus was obtained in the middle line. In agreement with this, the removal of one hemisphere through the plane of exit of the 3rd nerve did not abolish the effect obtained by excitation of the remaining hemisphere, although the arrest and acceleration effect disappeared.

The Degree of Stimulus.

8 and 6 may be taken as the usual amounts required. If the animal were in a very superficial stage of anæsthesia, less was needed. Currents of increasing strength up to 0 produced a similar but less marked result in the deeper stages of anæsthesia.

Causes which Prevented the Effect from being Obtained.

It disappeared in deep anæsthesia even with 0. As the animal became exhausted the effect was more and more incomplete.

Over-inspiratory clonus might be complicated with arrest or with over-inspiratory tonus. It was liable to be complicated with arrest when the cortex was excited, owing to spreading of the stimulus or the effect of the same from the junction of the olfactory bulb and tract to the adjacent arrest area. Over-inspiratory tonus tends

to affect the result in the sections behind the optic tract, where the strand passes ventral to the centre of the crusta. This was avoided by keeping the electrodes close to the under-surface of the crus, by not employing any stronger current than was absolutely necessary, and by using sufficient ether to diminish the tendency to over-inspiratory tonus.

Description of Tracings showing Over-inspiratory Clonus.

Tracing XIX., p. 646. Cat. Junction of olfactory bulb and tract, right side, see Photograph II. Over-inspiratory clonus was produced by 6 and 4, at first, also with 2, but afterwards arrest occurred from the extension of the stimulus to the arrest area. With this tracing may be compared the table on p. 623, as showing the effect of exciting the points marked 2, 3, 4, and 11 on Diagram I.

Tracing XX., p. 646. Dog. Junction of left olfactory bulb and tract, see Photograph III. The right hemisphere had been excluded by section between the plane of exit of the 3rd nerve and the anterior end of the pons. 8 produced the effect.

Tracing XXI., p. 647. Rabbit. Junction of left olfactory bulb and tract, see Photograph I*b*. The right hemisphere had been excluded by section at the plane of the optic tract. The clonus was twice elicited by a current of 0. The animal was in a deeper stage of anæsthesia than in the preceding cases. In a stage deeper still the reaction is lost.

Tracing XXII., p. 647. Dog. Tip of left uncinate convolution, see Photograph XI. and XII., and just external to the optic tract, after section of the right side between the plane of exit of the 3rd nerve, and the anterior end of the pons. The clonus was produced with 6.

Tracing XXIII., p. 647. Cat. The middle line at the ventral margin of the anterior end of the pons, see Photograph XV., after a right hemisection and removal of the hemisphere at this level, the clonus was produced with 10 and 8.

(3.) *Over-inspiratory Tonus.*

This is the most widely generalized effect of any stimulus upon the respiration. In a non-anæsthetized or partly anæsthetized animal, all previous observers have shown that a stimulus, if strong enough, will influence the respiration in this direction when applied to any sensory surface.

The greater relative influence of anæsthesia upon the over-inspiratory tonus has allowed of the other effects being largely or entirely freed from its complicating influence.

No doubt greater degrees of over-inspiratory tonus might be most easily produced on a non-anæsthetized animal, but all my experiments have been with anæsthetized animals ; and I may recall what I have mentioned before, viz., that there are other sources of anæsthesia than drugs, and in order to produce the same amount of over-

inspiration in the same animal with the same stimulus applied to the same place, it is necessary that the animal should be in a definite stage of anæsthesia, whether caused by ether, shock, hæmorrhage, &c. I have examined the production of over-inspiratory tonus from three points of view, by excitation of the 5th nerve and meninges, of the sciatic nerve after the removal of the cerebrum, as well as the cortical effects.

Over-inspiratory Tonus from Excitation of the 5th Nerve and Meninges.

In animals under sufficient ether to abolish voluntary movements, I only obtained by faradic excitation of the 5th nerve and meninges over-inspiratory tonus, and never observed any arrest of the respiratory rhythm. Slight excitation of the terminal ends of the 5th nerve in the nose, by chloroform or tobacco smoke, have been described as instances of the causation of arrest by excitation of this nerve. The well-known fact that every child or animal forced to inspire an anæsthetic, "holds its breath," is an example of this, and it requires a distinct effort of the adult human will to resist the tendency. This arrest through the peripheral terminations of the 5th nerve disappears when there is sufficient anæsthesia to abolish voluntary movements. Arrest has been produced in a like fashion by exciting the trunk of the nerve or its branches in the dura mater in a non-anæsthetized animal with a very weak current But an animal may be made "to hold its breath" by gentle excitation of many other sensory surfaces, such as the cerebral cortex or the sciatic nerve. In these cases, however, the arrest usually ends in a general convulsion, and on the repetition of such an experiment the convulsion is just as likely to occur primarily instead of an arrest.

When the anæsthesia is sufficient to abolish voluntary movements, then excitation of the 5th nerve root, of the Gasserian ganglion, of the divisions of the 5th nerve, and of the dura mater, results only in over-inspiratory tonus, and not in absolute arrest of the rhythm. If excessive currents are employed, the rhythm may be arrested by spreading from the dura mater to the arrest area of the cortex either immediately by direct contact, or mediately through the cerebrospinal fluid, or blood clot. It may also spread to the roots of the vagus. The amount of current required to produce over-inspiratory tonus varies with the amount of anæsthesia. In deep ether anæsthesia there was not the slightest effect produced upon the respiration even with the strongest current used. When the current was strong relative to the amount of anæsthesia, the thorax assumed an extreme degree of expansion, and the lever of the MAREY'S tambour no longer recorded anything but fine tetanic movements, but on direct observation the respiratory rhythm was seen to continue although small in amplitude and masked by the extreme expansion.

Description of Tracings from the 5th Nerve, showing Over-inspiratory Tonus.

Tracing XXIV., p. 651, Rabbit. Excitation of the 2nd division of the 5th nerve in the orbit. The result was over-inspiratory tonus, with a continuance of the rhythm.

Tracing XXIV.

Tracing XXVII.

14
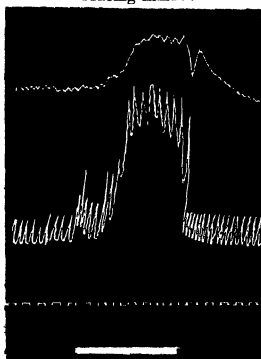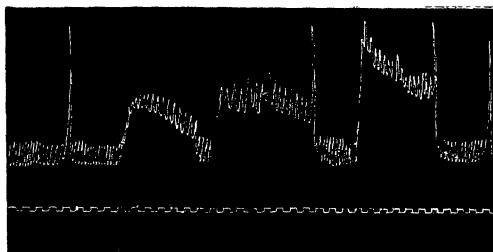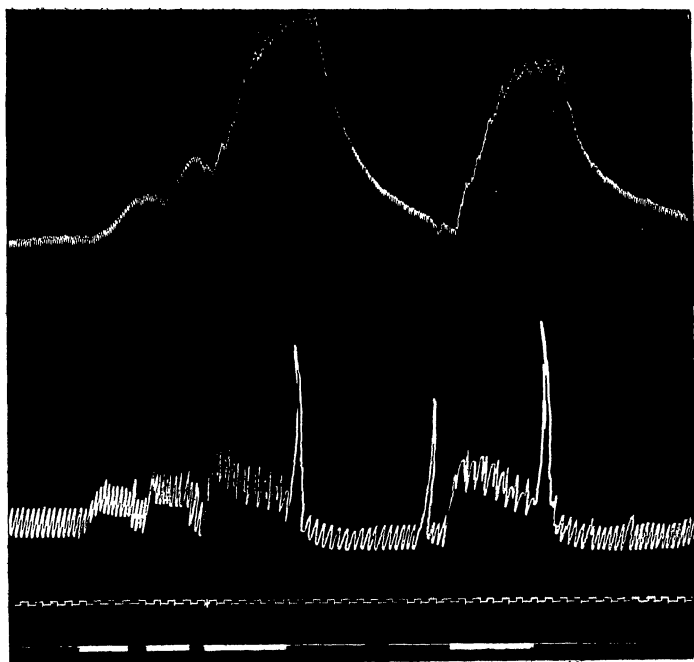
Tracing XXVI.

12   10

4 o 2

Tracing XXV., p. 647. Cat. Excitation of Gasserian ganglion. Marked over-inspiratory tonus occurred, and a diminution in amplitude from the expansion of the thorax which masked the respiratory movements.

In Tracings XXIV. and XXV. the same current was used ; and other tracings from the dog and monkey, with the same strength of stimulus, show similar effects, only rather less marked in the case of the monkey. In comparing a number of experiments upon the 5th nerve in the various species, it is to be noted that when the anæsthetic was insufficient acceleration of rhythm took place, and continued after the cessation of the stimulus, until the animal was given more of the drug, when, on repetition of the experiment, the rate was not altered. Also, in exciting the 5th nerve, or dura mater, near the olfactory bulb, care had to be taken that the current did not spread through the olfactory nerves to the bulb, and set up "clonus."

In deeper stages of anæsthesia the same result occurs, but a stronger excitation is required.

Over-inspiratory Tonus after Removal of the Cerebrum.

In order to show that over-inspiratory tonus is a general effect of exciting any sensory nerve, and has no special connection with the cerebrum, I excited the sciatic nerve after the complete removal of the cerebrum by incision at the level of the tentorium cerebelli.

Tracing XXVI., p. 651. Cat. Excitation of the left sciatic nerve in the popliteal space, after the removal of the whole of the cerebrum immediately above the pons.

Over-inspiratory tonus occurred with 12, 10, 8, and 6.

The Area on the Cortex where the most marked Over-inspiratory Tonus is produced, and the apparent course of the Fibres connecting this Area with the Medulla.

The greatest effect is to be obtained in the centre of the "sensori-motor" area. In the sections the best effect is obtained from the centre of the descending motor tract in the internal capsule, and lower down, in the middle of the crusta. These fibres do not decussate early, for if the opposite hemisphere be removed just behind the plane of exit of the 3rd nerve, the effect may still be obtained by stimulating their course downwards in the vertical sections of the remaining half.

Whether there is one spot in the sensori-motor area more easily affected than any other, I cannot say, so readily is the result influenced by the anæsthesia.

Tracing XXVII., p. 651. Cat. Excitation of the "sensori-motor" area.

Great over-inspiratory tonus was produced with 6, 4, and 2.

The same effect followed excitation of this area when the opposite hemisphere had been completely removed down to the tentorium cerebelli.

Tracing XXVIII., p. 646. Dog. Excitation of the crusta just behind the internal capsule, see Photograph XIII. 8 produced over-inspiratory tonus, the rhythm continuing.

Comparing a number of tracings of over-inspiratory tonus, I find that, although best marked in the sensori-motor area of the cortex, and in the line of the descending motor tract, yet it is produced outside this and complicates the "arrest" or "acceleration" effect, unless removed by the anæsthesia.

Thus, on the "arrest" area, arrest in over-inspiratory tonus follows excitation when the anæsthesia is insufficient. Conversely it may be obtained from the "arrest" area, uncomplicated by any arrest after opposite hemisection behind the anterior commissure, and from the "acceleration" area uncomplicated after a similar hemisection behind the third nerve exit.

Tracing XXV.

Tracing XXVIII.

14
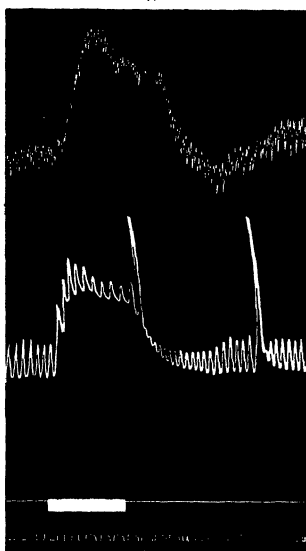
An Increased Action of the Respiration in the Monkey.

I have been forced to distinguish the monkey from the rabbit, cat, and dog, because in it an increased action of the respiration is so much less marked. There appears to be no fundamental difference, for I have obtained the same reactions, "acceleration," "over-inspiratory clonus," and "over-inspiratory tonus," at the points corresponding to those of the other animals, but only to a much smaller degree. The monkey reacts like the other three species do when very exhausted. I have not yet succeeded in artificially producing a greater excitability of the monkey's brain so

as to obtain marked increased action of the respiration. The result of such attempts has been to excite irregularity of the rhythm and general convulsions. This lessened representation of the other phenomena, or the relatively greater sensitiveness to the effects of anæsthesia on the part of the monkey, allows slowing and arrest to be obtained all the more readily, and the localisation of the points of representation to be more easily recognised, since the effect is not so liable to be complicated by a simultaneous calling forth of increased action.

On the cortex over-inspiratory tonus and acceleration are to be obtained at the anterior end of the sulcus known as X (sulcus frontalis superior).

A Note of the Concurrent Effects upon the Circulation.

The stimulus directed to producing respiratory changes sometimes influenced the circulation, and notably in two ways :—

(1.) A marked rise of blood pressure occurred, especially when the sensori-motor area of the cortex was excited and the sections in the course of the descending motor tract. But this happened only when the animal was not deeply anæsthetised. With deeper anæsthesia a very slight rise of blood-pressure took place, or no change at all.

(2.) In the region of the "arrest" area, especially in the neighbourhood of the sylvian artery, a fall of blood-pressure with a slower heart rate was met with.

But the exact conditions under which the circulatory effects can constantly be obtained has not yet been worked out.

CONCLUSION. (See Diagram II.)

It has proved itself an easy matter to fail in obtaining an effect upon the respiration by excitation of the cerebrum, either from experimenting upon inexcitable parts, or owing to excessive anæsthesia however produced.

Again, in all animals, when too slightly anæsthetized, complex and variable results are liable to occur, such as combinations of slowing and over-inspiratory tonus, acceleration changing to slowing, arrest interrupted by over-inspirations.

But, by careful regulation of the anæsthetic state, the following definite results follow constantly from faradic excitations. (See Diagram II.)

A. DIMINUTION OF ACTION.

Slowing and Arrest.

The cortical area, where this result was obtained, is situated just outside the olfactory tract in front of the point where the tract joins the temporo-sphenoidal lobe.

Followed back by vertical sections, the same result was obtained along the line of the strand of fibres known as the olfactory limb of the anterior commissure. After decussation in the latter structure, the tract continued backwards by the side of the infundibulum into the red nucleus below and external to the aqueduct in the plane of exit of the 3rd nerve.

Diagram II.

1. Cortical area for slowing and arrest.
2. Olfactory limb of anterior commissure.
3. Anterior commissure.
3(a). Fibres from the temporo-sphenoidal lobes.
4. Infundibulum.
5. Fibres passing backwards, causing slowing and arrest.
6. Red nucleus.
7. Exit of 3rd nerves.
8. Anterior fibres of pons.
9. Junction of olfactory bulb and tract.
10. Olfactory tract.
11. Uncinate convolution.
12. Uncus.
13. Connection of uncus and outer part of crus behind optic tract.
14. Optic tract and commencing optic radiation.
15. Tract passing obliquely beneath crusta.
16. The same, meeting the opposite one in the middle line at the anterior border of the pons.
17. Cortical area for acceleration of rhythm.
18. Tract external and ventral to internal capsule.
19. Tract passing below the junction of the internal capsule and crusta, above the optic tract, to the tegmentum.
20. Meeting of tract of either side in interpeduncular grey matter, at the level of and just behind the exit of the 3rd nerve.
21. Cortical areas for over-inspiratory tonus.
22. Tract passing through the motor portion of the internal capsule dorsal to the optic tract.
23. Tract running in the crusta.
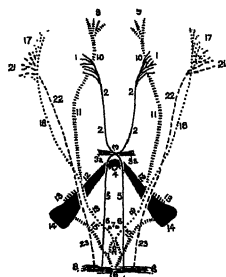
Slowing and arrest.
Acceleration.
Over-inspiratory clonus.
- - - - Over-inspiratory tonus.

B. INCREASED ACTION.

(1) *Acceleration.*

Commencing especially from a point on the convex surface of the cortex within the sensori-motor area, the effect may be followed back through the lenticular nucleus where it borders on the outer and ventral portion of the internal capsule; the strand runs at first externally and then ventrally to the motor portion of the internal capsule, and so reaches the tegmentum. The lines from the two sides meet in the inter-peduncular grey matter at the level of and just behind the plane of the 3rd nerves.

(2) *Over-inspiratory Clonus.*

This effect was obtained from the junction of the olfactory bulb and tract, and on continuing to apply the stimulus backwards along the olfactory tract was traced into the uncinate convolution of the temporo-sphenoidal lobe. Followed to the uncus it passed behind the optic tract to the crus, and then pointing obliquely inwards ventrally to the crusta the effect on each side converged to the middle line at the upper border of the pons.

(3) *Over-inspiratory Tonus.*

The descending motor tract yielded this result, so did the 5th nerve and dura mater, as well as the sciatic nerve after complete removal of the cerebrum at the level of the tentorium cerebelli.

So far as the anatomical arrangement of the tracts above described is concerned, I may say that medullated fibres are to be seen in Dr. TOOTH's preparations of the cat's and monkey's brain, running in the same course as that indicated by faradic excitation of living sections. An exception to this statement occurs in the case of the over-inspiratory clonus, in which I have not made out the connection between the uncus and the pontine fibres lying ventral to the crusta.

It is manifest that the respiratory alterations described above as capable of being induced by the faradic current are such as can be produced in the conscious state by volitional effort, and any explanation of the results described above must depend upon the acceptation or refusal of the general doctrine concerning the sensori-motor functions of the cortex, and that concerning the mechanism of the respiratory centre in the medulla.

I sum up therefore, the contents of this paper, when I say that whilst the effect upon respiration of exciting the cerebrum in a non-anæsthetized animal is probably a complex one, yet, by careful regulation of the anæsthetic state, four constant effects can be obtained upon respiration by stimulation of the cortex cerebri, and these can be traced down each in a course of its own from the cortex to the medulla oblongata.

DESCRIPTION OF PLATES.

PLATE 57.

Photograph IA.—Under-surface of a Rabbit's Brain, the small ring marks the point for slowing and arrest, the square that for inspiratory Tonus.
Photograph IB.—Dorsal surface of a Rabbit's Brain, the cross marks the point for acceleration.

Photograph II., with diagram.*—Ventral and lateral surface of a Cat's Brain, the inner ring marks the centre, the outer the boundary of the area for slowing and arrest.

Photograph III., with diagram*.—Ventral and lateral surface of a Dog's Brain, the inner ring marks the centre, the outer the boundary of the area for slowing and arrest.

PLATE 58.

Photograph IV., with diagram.*—Ventral surface of a Monkey's Brain, the inner ring marks the centre, the outer the boundary of the area for slowing and arrest

†Photographs V. to VIII.—Vertical transverse sections of Cat's Brain.

PLATE 59.

Photographs IX. to XV.—Vertical transverse sections of Cat's Brain.

* On the Diagrams appended to Photographs II., III., and IV., the Roman figures refer to the number of the tracings, and the dotted line from each number leads to the point of the brain, by excitation of which the tracing so numbered was obtained.

† The following marks have been placed on Photographs V. to XV., indicating the point on each half of the section by the stimulation of which was obtained:—

(1.) Slowing and arrest	Marked by a small ring.		
(2.) Acceleration	,,	,,	cross.
(3.) Over-inspiratory Clonus . .	,,	,,	square.
(4.) Over-inspiratory Tonus. . .	,,	,,	letter I.

Photograph 1 a
RABBIT'S BRAIN.

Photograph 1 b
RABBIT'S BRAIN

Photograph II
CAT'S BRAIN

Photograph III
DOG'S BRAIN

DIAGRAM
to Photo II

DIAGRAM
to Photo III

Photograph IV
MONKEY'S BRAIN

Photograph V
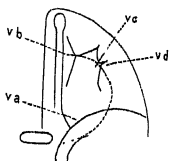
DIAGRAM
to Photo IV

Photograph VI

Photograph VII
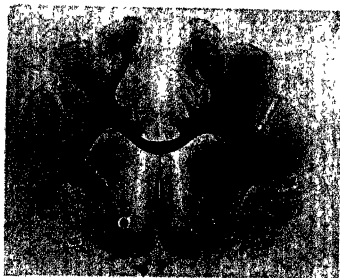
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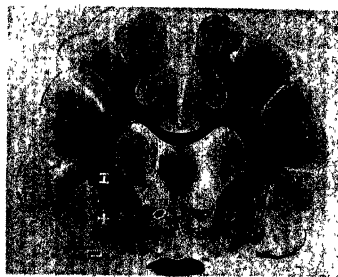
Photograph IX

Photograph X
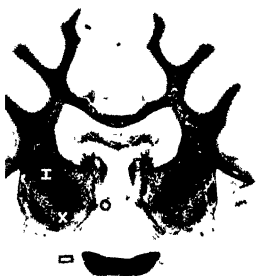
Photograph XI

Photograph XII

Photograph XIII

Photograph XIV

Photograph XV

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801. Part I...	0	17	0	1831. Part I...	1	10	0	1853. Part III...	1	2	0	1875. Part II...	3	(
— Part II...	0	17	6	— Part II...	1	12	0	1854. Part I...	0	12	0	1876. Part I...	2	
802. Part I...	0	11	0	1832. Part I...	1	1	0	— Part II...	0	16	0	— Part II...	2	
— Part II...	0	17	6	— Part II...	2	0	0	1855. Part I...	0	16	0	1877. Part I...	1	10
803. Part I...	0	12	6	1833. Part I...	1	1	0	— Part II...	1	6	0	— Part II...	2	
— Part II...	0	13	6	— Part II...	2	18	0	1856. Part I...	2	0	0	Vol. 168 (extra).	3	(
804. Part I...	0	10	0	1834. Part I...	0	17	0	— Part I...	1	4	0	1878. Part I...	1	10
— Part II...	0	12	6	— Part II...	2	2	0	— Part III...	1	4	0	— Part II...	3	(
805. Part I...	0	10	0	1835. Part I...	1	2	0	1857. Part I...	1	8	0	1879. Part I...	2	(
— Part II...	0	11	6	— Part II...	0	14	0	— Part II...	1	4	0	— Part II...	1	12
806. Part I...	0	13	6	1836. Part I...	1	10	0	— Part III...	1	2	0	1880. Part I...	2	
— Part II...	0	17	6	— Part II...	2	0	0	1858. Part I...	1	8	0	— Part II...	2	(
807. Part I...	0	10	0	1837. Part I...	1	8	0	— Part II...	3	0	0	— Part III...	1	1
— Part II...	0	15	0	— Part II...	1	8	0	1859. Part I...	2	10	0	1881. Part I...	2	10
808. Part I...	0	9	6	1838. Part I...	0	13	0	— Part II...	2	5	0	— Part II...	1	10
— Part II...	0	14	6	— Part II...	1	8	0	1860. Part I...	0	16	0	— Part III...	2	
809. Part I...	0	14	6	1839. Part I...	0	18	0	— Part II...	2	1	6	1882. Part I...	1	14
— Part II...	1	0	6	— Part II...	1	1	6	1861. Part I...	1	3	0	— Part II...	2	(
810. Part I...	0	10	6	1840. Part I...	0	18	0	— Part II...	1	5	0	— Part III...	2	10
— Part II...	0	12	0	— Part II...	2	5	0	— Part III...	1	7	6	— Part IV...	1	(
811. Part I...	0	15	0	1841. Part I...	0	10	0	1862. Part I...	2	14	0	1883. Part I...	1	10
— Part II...	0	15	0	— Part II...	1	10	0	— Part II...	3	0	0	— Part II...	2	10
812. Part I...	0	17	6	1842. Part I...	0	16	0	1863. Part I...	1	14	0	— Part III...	1	12
— Part II...	0	17	6	— Part II...	1	2	0	— Part II...	1	7	6	1884. Part I...	1	8
813. Part I...	0	14	0	1843. Part I...	0	10	0	1864. Part I...	0	11	0	— Part II...	1	16
— Part II...	0	18	0	— Part II...	1	10	0	— Part II...	1	7	6	1885. Part I...	2	10
814. Part I...	0	18	0	1844. Part I...	0	10	0	— Part III...	1	10	0	— Part II...	2	5
815. Part II...	1	2	0	— Part II...	1	10	0	1865. Part I...	2	2	0	1886. Part I...	1	8
816. Part II...	0	17	6	1845. Part I...	0	16	0	— Part II...	1	5	0	— Part II...	1	15
819. Part III...	0	10	0	— Part II...	1	0	0	1866. Part I...	1	14	0	1887. (A.)	1	3
823. Part II...	1	8	0	1846. Part I...	0	7	6	— Part II...	2	7	6	— (B.)	1	16
824. Part I...	0	12	6	— Part II...	1	12	0	1867. Part I...	1	3	0	1888. (A.)	1	10
— Part II...	1	0	0	— Part III...	1	12	0	— Part II...	1	15	0	— (B.)	2	17
— Part III...	1	4	0	— Part IV...	1	12	0	1868. Part I...	2	5	0	1889. (A.)	1	18
825. Part I...	1	4	0	1847. Part I...	0	14	0	— Part II...	2	0	0	— (B.)	1	14
— Part II...	2	0	0	— Part II...	0	16	0	1869. Part I...	2	16	0	1890. (A.)	1	16
826. Part I...	1	2	6	1848. Part I...	1	0	0	— Part II...	3	3	0	— (B.)	1	5
— Part II...	0	12	6	— Part II...	0	14	0	1870. Part I...	1	10	0	1891. (A.)	2	2
— Part III...	2	0	0	1849. Part I...	1	0	0	— Part II...	1	18	0	— (B.)	3	3
— Part IV...	1	2	6	— Part II...	2	5	0	1871. Part I...	1	10	0	1892. (A.)	2	1
327. Part I...	0	18	0	1850. Part I...	1	10	0	— Part II...	2	5	0	— (B.)	2	2
— Part II...	0	18	0	— Part II...	3	5	0	1872. Part I...	1	12	0	1893. (A.)	3	14
328. Part I...	1	1	0	1851. Part I...	2	10	0	— Part II...	2	8	0	— (B.)	2	15
— Part II...	0	10	0	— Part II...	2	10	0	1873. Part I...	2	10	0	1894. (A.) Pt. I.	1	5
329. Part I...	0	16	0	1852. Part I...	1	6	0	— Part II...	3	5	0	— (B.) Pt. I.	1	16
— Part II...	0	14	0	— Part II...	2	3	0	1874. Part I...	2	0	0			